CEREAL CHEMISTRY

Published bi-monthly by

The American Association of Cereal Chemists

W. F. GEDDES, Editor-in-Chief
R. J. TARLETON, Managing Editor } University Farm, St. Paul 1, Minn.

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Entered as second-class matter March 3, 1932, at the post office at Lancaster, Pa., under the Act of August 24, 1912. Acceptance for mailing at special rate of postage provided for in paragraph (d-2), Section 34.40, P. L. & R. of 1948, authorized February 16, 1924.

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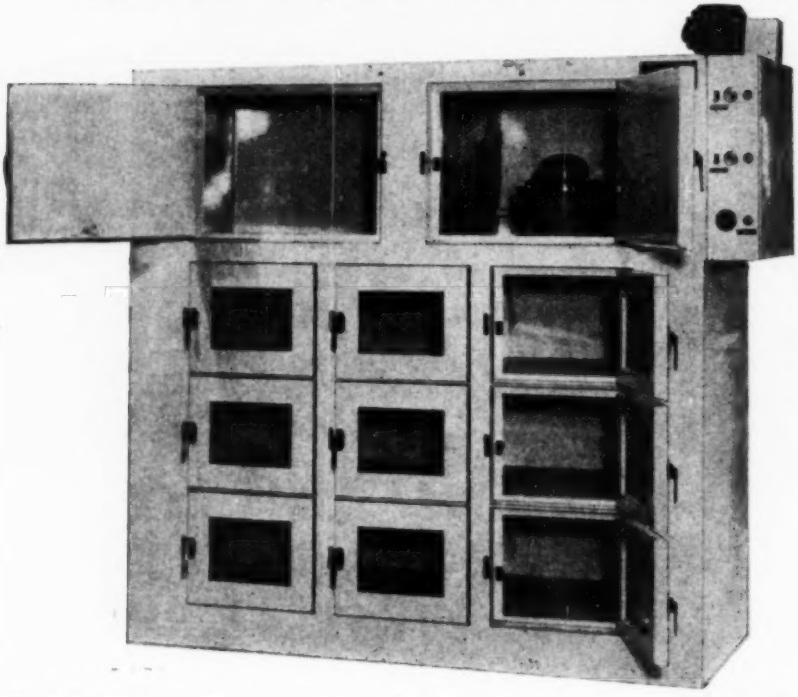

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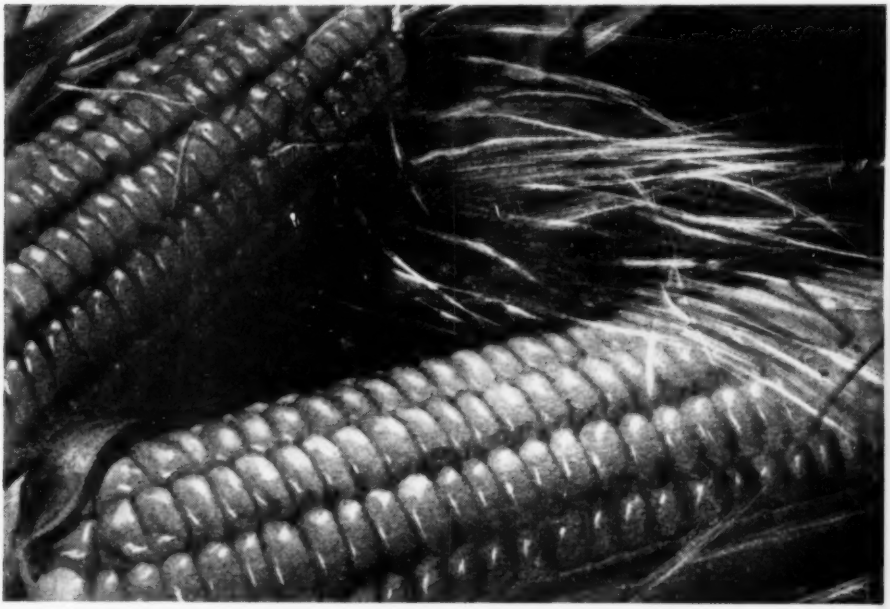

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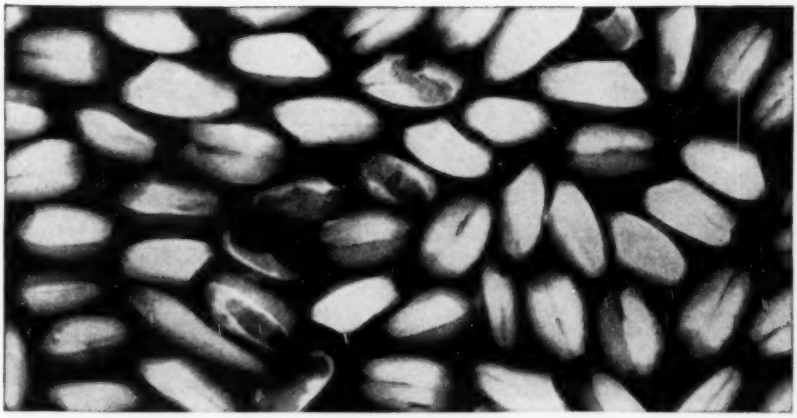

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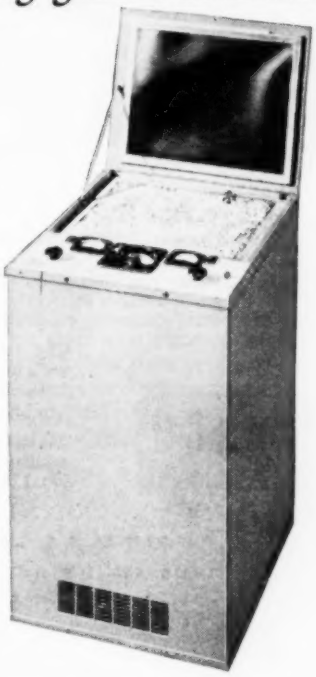

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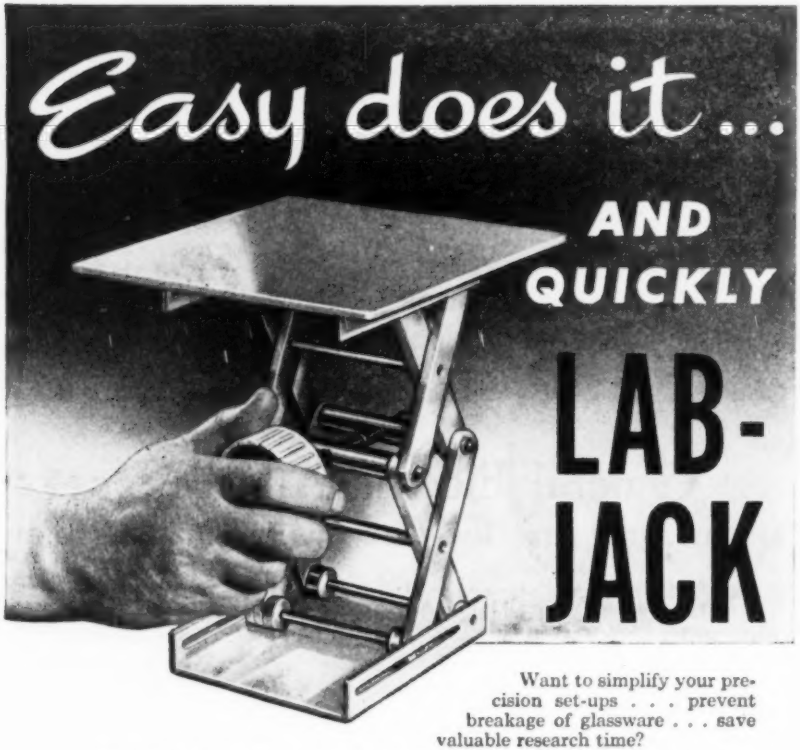

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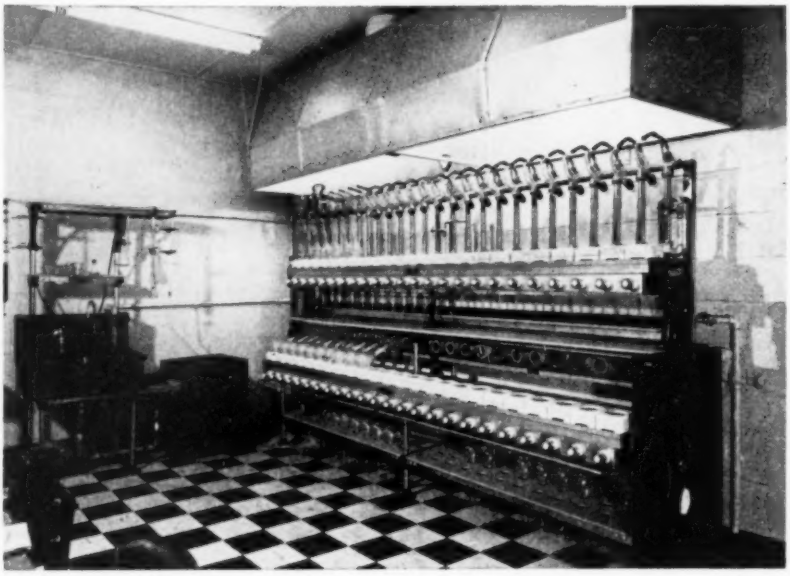

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CEREAL CHEMISTRY

VOL. XXIX JULY, 1952 NO. 4

RAPID DETERMINATION OF CRUDE FIBER IN CEREALS [1]

J. H. VAN DE KAMER and L. VAN GINKEL [2]

ABSTRACT

The crude fiber content of cereals may be rapidly and accurately determined by combining and modifying the procedures of Scharrer, Kürschner, and Von Fellenberg. The finely ground sample is digested for 30 min. with a reagent consisting of nitric acid and trichloroacetic acid dissolved in 70% acetic acid. This treatment completely solubilizes the starch, protein, and lignin as well as most of the pentosans but does not attack cellulose or fat. The fat is extracted from the digestion mixture by the addition of ethyl ether. The crude fiber is filtered and washed on a glass crucible; to assure a high speed of filtration, it is imperative to use a small quantity of crude fiber and a big filter surface. Next, it is dissolved in concentrated sulfuric acid and oxidized with dichromate. From the quantity of dichromate used, as determined by back titration with standard thiosulfate solution, the quantity of crude fiber is calculated.

Sixteen to 20 determinations per man-day can readily be made. With wheat products, especially, the results are in good agreement with various modifications of the customary Weende method which involves successive digestion of the ether-extracted material with 1.25% solutions of sulfuric acid and sodium hydroxide followed by the gravimetric determination of the organic residue.

The method is especially recommended for baby foods and other cereal products of low crude fiber content.

About the year 1900 Kellner (7) and others, on the basis of many feeding experiments with oxen, studied the relationship between the nutritive value of various foods and their crude fiber content, determined according to the Weende method.[3]

In judging the nutritive value of a product, the Weende method of Henneberg and Stohmann for the determination of the crude fiber content is still in general use, in spite of the many analytical objections accompanying the carrying out of this method.

The use of various other methods which, from an analytical point of view, all have one or more advantages over the Weende method has

[1] Manuscript received May 25, 1951.
[2] Central Institute for Nutrition Research T.N.O., Utrecht, The Netherlands.
[3] "Weende" method means the method of Henneberg and Stohmann (5) worked out and first applied in Weende, a small German village with a Station for Agricultural Research. In Holland the method is always called the "Weende" method.

been recommended, without, however, much success. The results always deviate from those obtained by the Wennde method because each digestion liquid produces a crude fiber of a different composition and, therefore, a different amount. Crude fiber is a complex mixture, composed chiefly of cellulose, hemicellulose, pentosans and lignin, each of which is affected in a different way by different means of digestion. Since the relation between the nutritive value and the crude fiber content of feeds has been established only for crude fiber data, determined according to the Weende method, the feed industry will not change over to a new method, even though it is quite certain that the new method is absolutely satisfactory analytically, especially with regard to reproducibility and speed. According to this view, adoption of any new method for crude fiber determination would make it imperative to carry out new animal experiments in order to be able to calculate the correlation between the analytical data yielded and the nutritive value.

In the authors' opinion, there is no need for such a conservative policy. In the first place, the accuracy of animal experiments is limited. Further, it is not known which modification of the Weende method Kellner used; this is of paramount importance since the data obtained in the Weende methods greatly depend on the technique followed.

The authors feel that a simpler and accurate method, giving results, varying from those of the Wennde method by not more than some 10 or 20%, would be acceptable without testing in time-consuming, expensive animal experiments.

Compare also in this connection the opinion of Thomas and Armstrong (13).

In Europe an attempt was made to introduce the method of Scharrer-Kürschner (10). In principle, this method was first brought forward by Von Fellenberg in 1918 (3) and modified later by Kürschner and Hanak (8) in 1930. Finally in 1932, Scharrer described the procedure which is most used today. Although, from an analytical point of view, this method certainly deserves preference over the Weende method. It is only necessary to boil once with the digestion liquid, the suspension does not bump during boiling, filtration proceeds more quickly than in the Weende method—the the process is still so complicated and so time-consuming that this improvement cannot be considered satisfactory. Von Fellenberg (4) has made another alteration whereby the determination is carried out titrimetrically on a semimicro scale. His figures, however, are not convincing and, moreover, it is very difficult to obtain reproducible results by the method he described.

However, with a few small alterations, the present authors found that the last modification of Von Fellenberg produced results that could be duplicated very well. The advantages are now plain: drying, ignition, and weighing are replaced by a quick digestion with dichromate followed by a sinple titration; also, because of the small quantity of material used, the filtration of the crude fiber proceeds easily. In this manner, it is possible to do one determination in about 1.5 hours, while a total of 16 to 20 determinations can be accomplished by one person per day. The method, as modified by the authors, is described in this paper.

Rapid Method for the Determination of Crude Fiber (Cellulose)

Principle of the Method.—The finely ground sample is digested by boiling for exactly 30 minutes with the reagent of Scharrer and Kürschner (nitric acid and trichloroacetic acid dissolved in acetic acid, 70%). Cellulose and fat are attacked to only a slight degree, pentosans dissolve almost completely while starch, protein, and lignin dissolve entirely.

After cooling, ether is added and the mixture filtered through a large filter crucible. By this means, fat and other substances soluble in the reagent are removed, while the cellulose and a small portion of the pentosans (<10%) remain on the filter. After washing with reagent, ether, reagent and water, concentrated sulfuric acid is poured over the cellulose on the filter. The cellulose dissolves and the solution is then drawn through the filter by suction and collected in a standard solution of dichromate in sulfuric acid. The mixture is then heated for 10 minutes in a boiling water bath, where the cellulose is oxidized to carbon dioxide and water. After dilution and addition of potassium iodide, the quantity of dichromate used is determined by back titration with standard sodium thiosulfate solution. From this, the number of mg. crude fiber ("cellulose") present in the starting material is calculated.

Two different concentrations of dichromate are used, depending on the crude fiber content of the starting material.

Reagents:

1. Scharrer-Kürschner reagent. Dissolve 50 g. trichloroacetic acid in 1 to 1.5-l. acetic acid (70%). Add 124 ml. nitric acid (65%, density 1.4) and make up to 2-l. with acetic acid (70%).
2. Potassium dichromate in sulfuric acid, approximately 0.2 *N*. Dissolve 2.45 g. potassium dichromate in 160 ml. water and add 120 ml. concentrated sulfuric acid solution (96%).
3. Potassium dichromate in sulfuric acid, approximately 1.2 *N*. Dissolve 14.7 g. potassium dichromate in 160 ml. water and add 120 ml. concentrated sulfuric acid solution (96%).
4. Sodium thiosulfate solution, 0.1 *N* (to be accurately standardized).
5. Potassium iodide solution, 1.0 *N*.

Apparatus:

1. Acetylation flasks, 150 ml. (see Fig. 1, A).
2. Air condensers.
3. Burner with glass chimney (see Fig. 1.C).
4. All glass Buchner-type funnel with fritted disc filter (see Fig. 2, D). (e.g. 17 G 3 Schott-Jena, diameter 65 mm.).
5. Erlenmeyer filter flasks, 500 ml.
6. Erlenmeyer filter flasks, 500 ml. (with bottom cut off and ground edge, see Fig. 2, C).
7. Glass plates ground flat (see Fig. 2, A).
8. Erlenmeyer flasks, 1-l. and 250 ml., with ground glass stoppers.
9. Erlenmeyer flasks, 100 ml. (see Fig. 2, B).

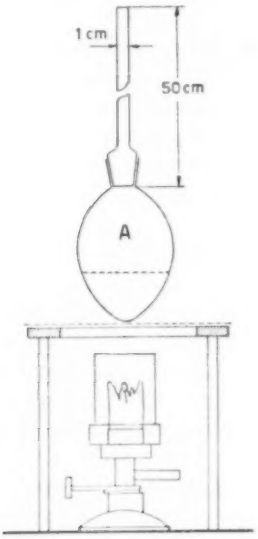

FIG. 1. Digestion assembly. A—Acetylation flask with air condenser having a ground glass joint.

a. Procedure for Samples with a Low Crude Fiber Content (up to 2%.—Grind the sample in a laboratory mill finely enough to pass through a sieve with openings of 0.5–0.8 mm. and weigh out an amount corresponding to 5–25 mg. crude fiber. Transfer the sample to an acetylation flask and add 8 times as much reagent as the weight of sample in grams, not less, however, than 10 ml. Bring the contents of the flask as quickly as possible to a boil. Under continual swirling, boil gently for exactly 30 minutes in a flask to which an air condenser with a ground glass joint has been attached (beware of scorching). Cool immediately in cold running water, add 1.5 times the volume of ether and swirl for a few seconds until two layers have clearly separated; filter the upper layer only through the Buchner funnel [4] Repeat this

[4] After the determination has been carried out, the Buchner funnel is washed a few times with water until the filtrate is acid-free. The crucible is then ready for the next determination.

process twice, swirl and filter off the ether layer; finally add 25 ml. cold reagent and filter the entire contents of the flask through the Buchner funnel. Wash with cold reagent until the filtrate no longer shows cloudiness on the addition of water; (usually washing 3 times with 10 ml. is sufficient). Remove the filtrate from the filter flask, replace the Buchner funnel and again wash 3 to 5 times with hot water until acid-free to litmus. Then place the filter crucible on an Erlenmeyer flask of 300 to 400 ml., with bottom cut off (see Fig. 2). Pipette 25 ml. of 0.2 *N* dichromate sulfuric acid solution into an 100 ml. Erlenmeyer flask and put this under the Erlenmeyer filter flask so that the stem of the Buchner funnel projects into the Erlenmeyer flask. Place 5 ml. conc. sulfuric acid on the filter and stir for 1 to 2 minutes until all the crude fiber is dissolved. Draw off the acid solution by suction

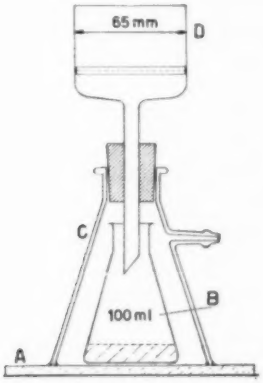

FIG. 2. Filter flask arrangement. A—Glass plate. B—Receiving flask. C—Filter flask with bottom cut off. D—Buchner funnel with fritted disc filter.

and wash 5 times with 5 ml. conc. sulfuric acid, the preceding 5 ml. always being completely removed before the following 5 ml. is added.

Place the Erlenmeyer flask containing the solution of crude fiber in dichromate-sulfuric acid for 10 minutes in a boiling water bath. After cooling, wash the contents with 500 ml. of water into a wide-necked Erlenmeyer flask with stopper (take care that the neck of the Erlenmeyer flask does not become wet), cool again and add, while swirling, 10 ml. 1.0 *N* potassium iodide solution by means of a pipette. After standing 10 minutes, titrate the excess dichromate with 0.1 *N* sodium thiosulfate using starch as an indicator.

Determine the result of a blank titration, namely, of: 25 ml. 0.2 *N* potassium dichromate-sulfuric acid + 30 ml. sulfuric acid, 96% + 500 ml. water + 10 ml. potassium iodide.

Calculation.

$$\% \text{ crude fiber} = \frac{(t_2 - t_1) \times N \times 6.86}{p} \times 100,$$

where: t_1 = ml. sodium thiosulfate used; t_2 = ml. sodium thiosulfate used by the blank; N = titer of the sodium thiosulfate; p = weight of sample in mg. and 6.86 = factor for calculating the cellulose content from the dichromate used.

b. Procedure for Samples with a Hiigh Crude Fiber Content (above 2%).—The determination of the crude fiber content of shorts, bran, etc., is carried out as follows:

Weigh a quantity of the ground sample which contains 25—150 mg. crude fiber. Carry out the determination as described above, but pipette 25 ml. of 1.2 *N* dichromate-sulfuric acid solution into the Erlenmeyer flask in which the cellulose dissolved in sulfuric acid is collected and wash after oxidation into a graduate flask of 500 ml.; cool, fill up to the mark and mix. In 50 ml. of this liquid, titrate the unused dichromate.

Determine the result of a blank titration, namely, of: 25 ml. 1.2 *N* potassium dichromate-sulfuric acid + 30 ml. sulfuric acid 96% + water in a 500 ml. graduate flask. Titrate 50 ml. of this after addition of 10 ml. potassium iodide.

Calculation.

$$\% \text{ crude fiber} = \frac{(t_4 - t_3) \times N \times 6.86}{p} \times 10 \times 100$$

where t_3 = ml. sodium thiosulfate used; t_4 = ml. sodium thiosulfate used by the blank; N = titer of the sodium thiosulfate; p = weight of sample in mg. and 6.86 = factor for calculating the cellulose content from the dichromate used.

Observations. The sample must previously be finely ground in order to prevent the crude fiber from dissolving too slowly in the concentrated sulfuric acid whereby charring would take place. A slight brown coloration does not appear to influence the results.

From Fig. 3, it is clear that the usual 30-minute boiling period with Scharrer-Küschner reagent is not a very happy choice, for deviations in this time cause differences in results. Less trouble would be experienced with a boiling period of, e.g., 2 hours, especially for the products with a relatively high crude fiber content. (The composition of the "crude fiber" thus appears to be dependent on the boiling period.)

It appeared, however, to be wise to keep the boiling period to 30 minutes, because in this way the crude fiber contents of wheat products agreed well with the values obtained by the Weende method (see

Table III), while the reproducibility of the results appeared to be very good (see Table II), provided the boiling lasted exactly 30 minutes and cooling in cold running water took place immediately afterwards.

The time necessary to bring the different samples to a boil with Scharrer-Kürschner reagent cannot have a disturbing influence on the results. The samples with a high crude fiber content are digested with a small amount Scharrer-Kürschner reagent and, therefore, can be brought to a boil very quickly. Although the samples with a low crude fiber content are digested with larger quantities of reagent and the time necessary to bring the mixture to a boil, therefore, longer, the

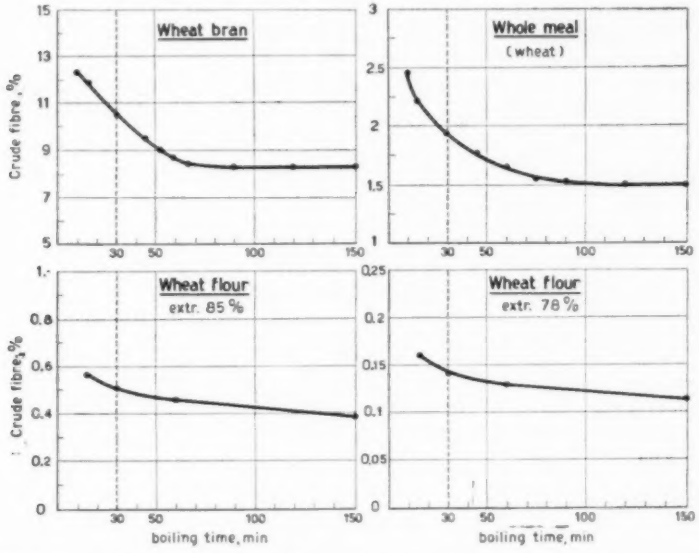

FIG. 3. The effect of digestion time with Scharrer-Kürschner reagent on the crude fiber values for different wheat products obtained by the titrimetric procedure.

crude fiber content is low and no disturbing influence on the results need be expected. This can be deduced from Fig. 3.

Washing with ether *after* the digestion has the advantage that previous defatting of a sample rich in fat is not necessary. Moreover, at first the heavy crude fiber remains at the bottom of the acetylation flask, so that the supernatant liquid can be filtered off very quickly through the filter, which has not yet become partly clogged by particles of crude fiber.

The use of a large filter crucible ensures a rapid filtration. The filter disc cannot become completely clogged since there is too little crude fiber present (Van de Kamer (6)). This is demonstrated by the

following results:

Sample Weight	*Jena Crucible*		*Crude Fiber in*	
g.	*No.*	*Duration of the Filtration*	*(a)*	*(b)*
7	11 G3	About 2 hours	2.01	1.99
7	17 G3	About 0.5 hours	1.97	1.96
0.5	17 G3	1 to 2 minutes	2.01	2.02

It is necessary to use filter crucibles with a tube because the usual rubber fastening of a filter crucible without a tube leads to errors owing to the concentrated sulfuric acid attacking the rubber.

If the crucible is immediately washed with water after filtering off the digestion mixture, then the duration of the filtration might be unfavorably influenced because in this way precipitates might form in the filter disc.

The principle of dissolving the crude fiber in concentrated sulfuric acid is taken from various procedures for the determination of cellulose (e.g. Tyden) (14).

At first, attempts were made to carry out the oxidation with dichromate-sulfuric acid on the filter (glass filter crucible or asbestos). It appeared, however, that this caused irregular results, since the filter material also modifies the dichromate-sulfuric acid. On page 116 of the catalogue "Jenaer Glas Für Laboratorien Cat. No. 5700" a serious warning is given concerning the use of dichromate-sulfuric acid with a glass filter disc. The chromic salts which are formed attach themselves very firmly to the glass filter disc, with the result that it is impossible to collect all the dichromate in the Erlenmeyer flask; hence the titration would be too low. This may be the reason why reproducible results were not obtained in applying the modification of Von Fellenberg (1932).

Ten minutes heating in a boiling water bath with dichromate-sulfuric acid of the given strength appears to be sufficient for oxidation of the crude fiber to carbon dioxide and water. This is shown by the following data:

	Duration of Oxidation in Min.			
Samples	*1*	*5*	*10*	*15*
Baby food	0.15	0.17	0.18	0.18
		0.16	0.17	0.16
Whole wheat meal, A	1.74	2.00	1.99	2.01
		1.94	1.97	1.98
				2.01
Whole wheat meal, B	1.86	1.97	2.04	2.07
	1.89	2.04	2.04	2.07
	—	1.96	2.03	—
	—	1.90	2.06	—
Wheat bran	10.0	10.3	10.9	10.8
	10.3	10.5	10.7	11.0

Results and Comparison of Different Methods

To determine whether or not different results are obtained by the application of titrimetic and gravimetric methods, after digestion according to Scharrer-Kürschner, different wheat products were investigated. The titrimetric method described above was followed and also the original gravimetric method of Scharrer and Kürschner (10), as modified by Thaler and Malzer (12), namely, filtration of the digest through filter paper followed by drying, weighing, ashing and reweighing, with a correction for the weight of the filter paper washed beforehand with Scharrer-Kürschner reagent.

TABLE I

COMPARISON OF TITRIMETRIC AND GRAVIMETRIC METHODS FOR THE DETERMINATION OF CRUDE FIBER IN WHEAT PRODUCTS

	Gravimetric			Titrimetric		
	Sample Weight	Crude Fiber		Sample Weight	Crude Fiber	
	g.	%	%	g.	%	%
Wheat flour I	30	0.20	0.20	5	0.20	0.21
					0.20	0.19
Wheat flour II	30	0.27	0.29	5	0.27	0.28
		0.28	0.29		0.28	0.29
Wheat flour III	10	0.56	0.57	2	0.56	0.56
Wheat flour IV	15	0.58	0.59	2	0.60	0.60
Wheat flour V	10	0.78	0.77	1.5	0.76	0.76
					0.75	0.76
Wheat flour 85% extraction	30	0.53	0.52	2.5	0.50	0.51
Whole wheat meal	10	2.02	2.03	0.5	1.93	1.92
Wheat bran (coarse and fine)	3	9.4	9.2	1	9.5	9.5
Wheat shorts	5	7.9	8.1	1	7.8	7.9
Wheat bran (fine)	3	10.5	10.7	1	10.8	10.7
Wheat bran (coarse)	3	10.8	10.9	0.5	10.6	10.8

The results obtained by these two methods were identical (Table I). To determine whether or not the oxidation of the crude fiber proceeds quantitatively, the following experiment was conducted.

Wheat bran was boiled with the Scharrer-Kürschner reagent and the crude fiber was separated and dried. One hundred twenty mg. of this was weighed in quadruplicate, placed on a 17 G3 filter crucible, dissolved in conc. sulfuric acid and, after collecting in dichromate, oxidized. Of the quantities weighed out, 100.5, 98.1, 100.1 and 99.3% crude fiber were respectively recovered, thus proving that by applying the oxidometric method, using the factor 6.86, the crude fiber is determined quantitatively. According to Segal (11), the factor 6.86 is more accurate than the theoretical factor 6.75, because the cellulose is not quantitatively oxidized to carbon dioxide and water.

The theoretical factor 6.75 is based on the following equation:

$$C_6H_{10}O_5 + 6\,O_2 \qquad 6\,CO_2 + 5\,H_2O$$

from which it follows that 1 ml. *N* thio is equivalent to 6.75 mg. cellulose.

The reproducibility of the results obtained by the titrimetic method is indicated by the data in Table II for replicate deetrminations of the crude fiber content of three wheat products.

An investigation was also made to ascertain to what degree the results obtained by the titrimetric method agreed with those obtained according to the Weende method. Different modifications of the latter method of procedure were applied, viz. the method prescribed by the Food Laws (15) in the Netherlands, the method of the National Agricultural Experimental Station in Maastricht according to the modification of Campen (2), the method of the A.O.A.C. (1) and also the modification of the Weende method according to Puranen-Tomula (9).

TABLE II

REPRODUCIBILITY OF CRUDE FIBER DETERMINATIONS BY THE TITRIMETRIC METHOD

Sample	No. of Determinations	Crude Fiber			Standard Error[1]
		Mean	Min.	Max.	
		%	%	%	%
Whole wheat meal	10	1.91	1.82	1.94	0.035
Wheat meal, 85%	16	0.50	0.47	0.51	0.013
Wheat bran	14	10.6	10.5	10.8	0.10

[1] The standard errors reported in this column are the values for a single determination.

Under the Dutch Food Laws, 2.5 to 5 g. of the ground sample is extracted on a water bath with a mixture of 50 ml. of ethanol and 25 ml. ether by boiling with 200 ml. of 1.25% sulfuric acid solution for one-half hour at a constant volume. The sample is filtered hot and washed with boiling water. The washed residue is boiled for one-half hour with 200 ml. of 1.25% sodium hydroxide solution, filtered, washed with boiling water until neutral, washed with ethanol and then with ether. The residue is then dried in a platinum crucible at 110°–120° C. and weighed before and after ignition. The loss in weight is calculated as crude fiber.

To speed up the filtration, Campen introduced a Jena G 1 crucible with a sintered glass disk covered with a layer of sand, a perforated porcelain disk and a second layer of sand. The use of an acetone sulfuric acid (1.25%) wash after the first hydrolysis also speeds the filtration.

Puranen-Tomula refluxed 3 g. of the ground sample with 200 ml. of a 1.25% solution of sulfuric acid for one-half hour. After a slight

TABLE III

COMPARISON OF THE RESULTS OF DIFFERENT METHODS FOR THE DETERMINATION OF CRUDE FIBER

Sample	Author's Method (modification of the method of Scharrer, Kürschner, and Von Fellenberg)		Weende Method (gravimetrically, 3 g. analyzed)			Method according to Puranen-Tomula (3 g. analyzed)	
			Modif. Dutch Food Law	Modif. of Agric. Expt. Station Maastricht	Modif. A.O.A.C.	Titrimetrically	Gravimetrically
	%		%	%	%	%	%
Patent wheat flour	0.96	(5.0)[1]	—	0.22	—	—	—
Straight wheat flour, extraction 78%	0.15	(2.5)	—	—	—	0.45	—
Straight wheat flour, extraction ca. 80%	0.157	(5.0)	—	0.22	—	—	—
Straight wheat flour, extraction 85%	0.50	(2.5)	0.54	0.48	—	0.54	0.66
Whole wheat flour	1.91	(0.5)	—	2.2	2.1	2.3	2.1
Wheat shorts A	7.4	(0.5)	—	7.3	—	—	—
Wheat shorts B	7.9	(0.5)	8.0	8.2	7.6	—	—
Wheat bran (coarse and fine)	9.5	(0.5)	9.1	9.5	8.4	—	—
Wheat bran (coarse)	10.6	(0.5)	10.7	11.1	10.3	11.8	11.7
Wheat bran (fine)	10.8	(0.5)	10.1	10.5	10.1	—	—
Corn flour, extraction 100%	1.09	(0.5)	—	1.20	—	—	—
Rolled oats	1.27	(0.5)	1.00	1.25	0.99	—	—
Rye flour middlings	6.0	(1.0)	—	5.8	—	—	—
Buckwheat "middlings" (feed)	8.0	(1.0)	—	11.8	—	—	—
Barley offals	15.7	(1.0)	—	14.4	—	—	—
Oat millfeed A	23.8	(0.5)	—	25.3	—	—	—
Oat millfeed B	24.5	(0.3)	27.5	27.9	26.8	—	—

[1] Brackets = grams analyzed.

cooling, 20 ml. of 28% potassium hydroxide solution was added by means of a pipette. After refluxing for one-half hour, cooling, filtering on asbestos, washing with hot water, with 1.25% acid, again with hot water and finally with alcohol and ether or acetone, the residue was dried at 105° C. and ignited. By adding a large quantity of potassium hydroxide, the solution becomes alkaline and the time-consuming acid filtration is avoided.

The results in Table III show that the titrimetric procedure described in this paper gives crude fiber values for wheat products which are in good agreement with those obtained by the method of Weende. However, in the case of products with a very low crude fiber content, the results obtained by the titrimetric method do appear to differ considerably from those of the Weende method. However, it seems very probable that the titrimetric results are the more correct. With samples of low fiber content, it is practically impossible to obtain accurate results with the gravimetric method of Weende, since the difference between two weighings may only be about 7 mg. crude fiber. Therefore the titrimetric method is especially useful for the investigation of baby foods.

In the examination of cattle foods which contain much lignin, the results of the Weende method and those of the titrimetric method of Scharrer-Kürschner will indeed differ, because on digestion with acid and alkali the lignin does not go into solution, which would be the case using the Scharrer-Kürschner reagent.

Literature Cited

1. Association of Official Agricultural Chemists, Official Methods of Analysis, 7th ed. Washington, D. C. pp. 346–347, 22.29–22.31. Crude fiber—Official. 1950.
2. Campen, W. A. C. De bepaling van het gehalte aan ruwe celstof volgens de Weender-methode in veevoeders. Landbouwkund. Tijdschr. **61:** 785–792 (1949).
3. Fellenberg, Th. von. Eine Oxydationsmethode zur Bestimmung der ligninfreien Rohfaser in Kakao. Mitt. Gebiete Lebensm. Hyg. **9:** 277–283 (1918).
4. Fellenberg, Th. von. Rohfaserbestimmung auf mikrochemischen Wege. Mitt. Gebiete Lebensm. Hyg. **21:** 385–390 (1930).
5. Henneberg, J. W. J. und Stohmann, F. Das Weenderverfahren. Beiträge zur Begründung einer rationellen Fütterung der Wiederkäuer, 1 und 2. Braunschweig 1860 und 1864 (cf. Böhmer, A., Juckenack, A., and Tillmans, J. Handbuch der Lebensmittelchemie, vol. 2 Allgemeine Untersuchungsmethoden, Berlin, p. 940, 1935).
6. Kamer, J. H. van de. De bepaling van ruwe celstof. Bakkerijwetenschap **2:** 26–30 (1949).
   Kamer, J. H. van de. La détermination de la cellulose brute. Bull. Ecole Meunerie Belge **10:** 105–112 (1948).
7. Kellner, O. Die Ernährung der landwirtschaftlichen Nutztiere. 8. Aufl. Paul Parey, Berlin (1919).
8. Kürschner, K., and Hanak, A. Zur Bestimmung der sog. Rohfaser. Ein neues Verfahren der Bestimmung der Rochellulose in Kakao. Z. Untersuch. Lebensm. **59:** 484–494 (1930).

9. PURANEN, U. H., and TOMULA, E. S. Ein schnelles und praktisches Verfahren zur Bestimmung der Rohfaser. Acta chemica fenmica **3:** 85–89 (1930) (ref. C. A. **25:** 2208 (1931).
10. SCHARRER, K., and KÜRSCHNER, K. Ein neues, rasch durchführbares Verfahren zur Bestmmung der Rohfaser in Futtermitteln. Bredermanns Lenbr. B. Tierernährung **3:** 302–310 (1932).
11. SEGAL, L., TRIPP, RITA C., TRIPP, V. W., and CONRAD, C. M. Determination of cellulose by acid-dichromate oxidation. Anal. Chem. **21:** 712–718 (1949).
12. THALER, H., and MALZER, A. Zur Bestimmung der Rohfaser nach K. Scharrer. Z. Untersuch. Lebensm. **83:** 141–144 (1942).
13. THOMAS, B., and ARMSTRONG, D. G. Crude fiber in feedingstuff analysis. Agriculture **57:** 210–211 (1950).
14. TYDÉN, H. Über die Bestimmung von α-Zellulose. Ing. Vetenskaps Akad. Handl. Nr. **175:** 1–83 (1943).
15. WARENWET, SPECERIJENBESLUIT. 1924, gewijzidg 1946 (Dutch Food Law, Spices Regulation, 1924. rev. 1946).

STUDIES ON SOME ASPECTS OF FLOUR IMPROVEMENT [1,2]

J. HOLME [3] and E. Y. SPENCER [4]

ABSTRACT

A titrimetric method using *o*-iodosobenzoate was employed to determine the effectiveness of potassium bromate and ammonium persulfate in oxidizing sulfhydryl groups from different flour fractions. These reagents caused no apparent reduction in the sulfhydryl content of both gluten washings and flour slurries when held at room temperature. Added glutathione however was partially oxidized.

The improvers studied in baking tests with different grades of flour increased effectiveness, although not uniformly, in the order persulfate, iodate, bromate and iodosobenzoate. The deleterious action of both glutathione and cysteine on loaf volume from a first clear flour was overcome to a greater extent (although not entirely) with bromate than with persulfate. A top patent flour showed no decrease in loaf volume with the addition of cysteine while a reduction in loaf volume again occurred with the addition of glutathione.

The phenomenon of flour bleaching or aging has long been a subject for much research and speculation. Although the characteristics of "green" and "aged" flours are easily recognized and can be altered by the use of suitable reagents, the mechanism of flour improvement is still unknown.

A number of different theories have been proposed to explain the effect of flour improvers on baking quality. Jorgenson (11) and Balls and Hale (3) emphasized the possible effect of oxidizing and reducing

[1] Manuscript received December 7, 1951.
[2] Contribution from the Department of Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan, with financial assistance from the National Research Council and the Saskatchewan Agricultural Research Foundation. Published as paper No. 296 of the Associate Committee on Grain Research of the National Research Council.
[3] Holder of an N.R.C. Bursary. Present Address: University of Minnesota, St. Paul.
[4] Present Address: Science Service Laboratory, University of Western Ontario Sub P.O., London, Ontario.

agents, such as bromate and glutathione, on the proteinases in wheat flour. In 1940 Sullivan and her co-workers (16) introduced the "direct-action theory of flour improvement," which emphasized the oxidation-reduction reactions of sulfhydryl groups in flour. More recently the results of Hlynka *et al.* (7, 9, 10) illustrate that a reaction occurs during storage between the protein and carbohydrate components of flour. Hlynka has suggested that this combination may, in part, be a cause of flour maturing.

The presence and possible significance of the sulfhydryl groups in flour have been investigated by such workers as Sullivan (16), Baker, Parker and Mize (2) and Myers and Working (13). The chief difficulty has been to find a suitable method for the reliable determination of the sulfhydryl content of flour and flour extracts, since these groups occur in only very small amounts. Hellerman, Chinard and Ramsdell (6) reported the use of sodium *o*-iodosobenzoate as a reagent specific for the determination of sulfhydryl groups in urease. McDonald (12) and Sullivan (15) found that this reagent was a powerful flour improver when incorporated into the standard baking formula.

Because of this specificity and observed effect on loaf volume the use of *o*-iodosobenzoate as a reagent for quantitatively determining the sulfhydryl content of flour systems seemed promising. Nordin and Spencer (14) adapted the use of *o*-iodosobenzoate to a radioactive method for sulfhydryl determinations, in which radioactive iodine, I^{131} was employed as an indicator of the endpoint. The results indicated that an unimproved first clear flour milled from Canadian hard red spring wheat contained in the order of 1.2×10^{-6} equivalents of sulfhydryl groups per gram of flour.

The present report deals with the application of a titrimetric method of using *o*-iodosobenzoate for assessing the effectiveness of potassium bromate and ammonium persulfate in oxidizing sulfhydryl groups of different flour fractions. This titration procedure was also applied in studying differences in the availability of the sulfhydryl groups. The relative effects of certain oxidizing and reducing agents, particularly ammonium persulfate, potassium bromate and *o*-iodosobenzoate, on loaf volume using different grades of flour were also investigated.

Materials and Methods

Three flours milled from Canadian hard red spring wheat were employed, namely, an untreated commercially milled first clear, an untreated commercially milled baker's patent and an experimentally milled straight grade flour from Thatcher wheat of 13.0% protein content.

The method of Hellerman *et al.* (6) using *o*-iodosobenzoate involved

adding a small excess of this reagent to the system, and determining the excess by addition of acid potassium iodide with standard thiosulfate solution. Preliminary experiments indicated a serious source of error arising from the reaction of the free iodine with the flour proteins. To alleviate this difficulty a known volume of standard thiosulfate was added before the acid potassium iodide. In this way the released iodine reacted with the thiosulfate. The excess thiosulfate was titrated with standard iodine solution to a starch endpoint.

In addition to this difficulty there is the apparent one involving adsorption of the *o*-iodosobenzoate upon the flour particles and its removal from the reaction medium upon centrifugation of the slurry. This error would cause results to be high. However, a comparison of the results with those from the radioactive iodine method (14) (where adsorption is eliminated) indicated that adsorption was insufficient to interfere with the value of the method as a measure of change in sulfhydryl content.

Flour-water slurries (1:4) parts were prepared with the straight grade flour, and the sulfhydryl content of the slurry determined by the following method. Ten grams flour, 38.2 ml. of water and 1.80 ml. of 0.0213 *N* sodium *o*-iodosobenzoate solution were stirred in a 100 ml. centrifuge tube for three minutes. The mixture was centrifuged at 2000 r.p.m. for five minutes and the volume of the clear supernatant measured. To this were added 2.0 ml. of 0.0247 *N* thiosulfate solution, 1.0 ml. of 20% acetic acid, 5.0 ml. of 2.5% potassium iodide solution and 2.0 ml. of starch indicator solution. After one minute the excess thiosulfate was titrated with standard iodine solution (0.0126 *N*).

Gluten washings were prepared by centrifuging the washings (about 1500 ml.) obtained upon kneading a flour-water dough containing 100 g. of flour.

For the sulfhydryl determinations, 100 ml. aliquots of the washings were analyzed by a method similar to that for slurries except that, because of the lower sulfhydryl content, reagents of about one-tenth concentration were employed.

The water-soluble proteins contained in the gluten washings were removed by saturating 100 ml. aliquots of the washings with solid ammonium sulfate. After one hour, the filtrates were collected through No. 40 Whatman filter papers and their sulfhydryl contents determined by the method used for gluten washings.

Basic-formula doughs were prepared with the baker's patent flour according to the standard A.A.C.C. method (4). Dough washings were collected and sulfhydryl contents determined by methods similar to those for gluten washings.

The effect of potassium bromate and potassium persulfate upon the sulfhydryl contents of gluten washings, protein-free washings and dough washings was studied by addition of these reagents to the systems.

The effect of oxidizing agents on the sulfhydryl content of systems containing added glutathione was studied. Stock solutions of glutathione containing 0.400 g./liter and of potassium persulfate containing 0.1352 g./liter were prepared. Gluten washings were collected from the baker's patent flour and aliquots were treated with known volumes of glutathione and persulfate solutions. The sulfhydryl content was determined over a period of reaction time.

Baking experiments were carried out with the object of determining the effect of a number of flour improvers and of some reducing reagents on loaf volume. All bakes were made using a semi-rich formula. The chemical reagents used in the various treatments were added at the mixing stage.

Baking Formula:

100 g. flour	3 g. hydrogenated vegetable shortening
3 g. yeast	4 g. skim milk powder
5 g. sugar	0.1 g. ammonium dihydrogen phosphate
1.25 g. sodium chloride	

In one series of baking tests, increasing amounts of *o*-iodosobenzoate, persulfate and a mixture of these two substances were added to doughs made with the first clear flour.

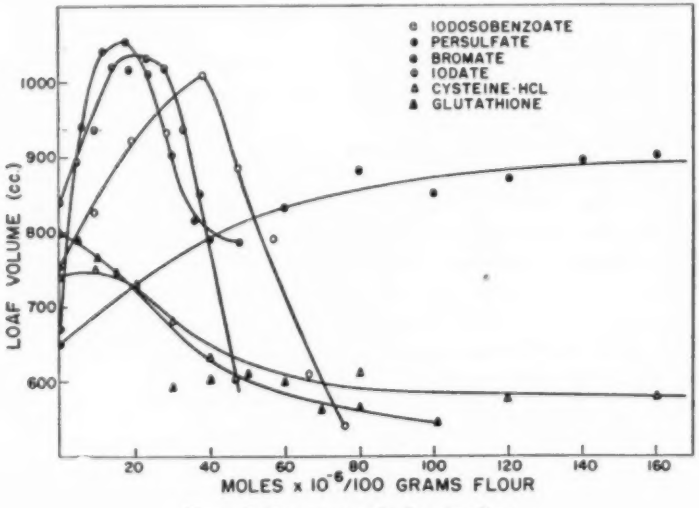

FIG. 1. Baking response of a first clear flour.

In another series, this flour was treated similarly with a wide variety of reagents, as will be shown in Fig. 1. In a third series, doughs made from the first clear flour were treated with the amount of potassium bromate (18×10^{-6} mole per 100 g. flour) required to produce maximum volume, plus increasing amounts of cysteine hydrochloride, as well as with excess bromate (36×10^{-6} moles) plus increasing amounts of glutathione (GSH).

In a further series, the baker's patent flour was treated with a wide variety of reagents and with combinations of oxidizing and reducing reagents. In one case the doughs were treated with 50×10^{-6} moles/100 g. flour of GSH (an amount which produced a very marked decrease in loaf volume) plus increasing amounts of potassium bromate. In other experiments ammonium persulfate was used in place of potassium bromate.

In addition doughs made with the first clear flour were treated with 19×10^{-6} moles of bromate/100 g. flour (a definite excess) plus increasing amounts of GSH.

Results

The sulfhydryl content of flour slurries made from the experimentally-milled straight grade flour was $1.34 \pm 0.04 \times 10^{-6}$ equivalents (moles) of sulfhydryl per gram of flour.

TABLE I

SULFHYDRYL CONTENT OF GLUTEN WASHINGS AND PROTEIN PRECIPITATED WASHINGS AND THE EFFECT OF OXIDANTS

Flour Sample	System Analyzed	Equiv. SH $\times 10^6$/gm. Flour
I. Composite Straight	—gluten washings	0.41
	—protein precipitated gluten washings	0.31
	—1 ml. persulfate (0.001 *N*) per 100 ml. washings for 1 hour at room temp.	0.41
	—2 ml. persulfate as above	0.41
	—5 ml. persulfate as above	0.40
II. Commercial Unimproved Top Patent	—gluten washings	0.49
	—1 ml. persulfate (0.001 *N*) per 100 ml. washings for 1 hour at room temp.	0.50
	—1.5 ml. persulfate as above	0.50
III. Freshly Milled First Clear	—gluten washings	0.58
	—protein precipitated gluten washings	0.29
	—protein precipitated washings 1 ml. bromate (0.01 *N*) at room temperature	
	30 minutes	0.26
	60 minutes	0.26
	120 minutes	0.27

Table I gives the results of sulfhydryl determinations on gluten washings and protein-free washings along with the effect of oxidizing agents on the values. Corresponding data for the dough washings are given in Table II.

TABLE II

SULFHYDRYL CONTENT OF DOUGH WASHINGS

Flour	Dough Treatment	Equivs. SH $\times 10^6$/gm. Flour
I. Commercial top patent SH content of gluten washings = 0.49×10^6 equivs/gm.	—washed immediately after mixing	0.52
	—washed following 3 hours fermentation	0.66
	—4 ml. $KBrO_3$ (0.02 *N*) included in dough. Washed after 3 hours fermentation	0.68

The reaction of glutathione with potassium persulfate in gluten washing media is illustrated in Table III. Whereas in Tables I and II the oxidizing agents did not decrease the SH contents of the systems, in Table III it is shown that persulfate can oxidize the sulfhydryl groups of added glutathione.

TABLE III

REACTION OF GLUTATHIONE WITH POTASSIUM PERSULFATE IN GLUTEN WASHING MEDIA

Experiment	Time	SH Content of 100 ml. Aliquot of Washings. (Equiv. $\times 10^{-6}$)
I. 100 ml. washings	—	2.82
II. 100 ml. washings		
3 ml. persulfate	0	2.80
	30	2.89
	60	2.81
	90	2.78
III. 100 ml. washings		
5 ml. glutathione	—	9.28
IV. 100 ml. washings		
5 ml. glutathione		
5 ml. persulfate	0	9.34
	30	6.49
	60	4.24
	90	3.60

The results of the baking experiments are represented graphically as loaf volume vs. moles $\times 10^6$ of reagents/100 g. flour. The influence of flour storage time on iodosobenzoate response has already been reported (14) and parallels that of standard maturing agents. In the first series of baking tests there was a marked difference in the effects of persulfate and iodosobenzoate. A maximum response to iodosobenzoate was obtained with 39×10^{-6} moles/100 g. flour, while in the range of treatment up to 60×10^{-6} moles/100 g. flour, persulfate produced no maximum in loaf volume, thus differing from the results of Doty and Sherwood (5) which suggest a maximum loaf volume with about 40×10^{-6} moles of persulfate.

The results of the second series, illustrated in Fig. 1, show the diverse effects of various reagents. Iodosobenzoate, bromate and iodate produced initial marked increases in loaf volume, followed by rapid decreases, whereas cysteine and glutathione caused decreases in loaf volume throughout the range of treatment. Potassium persulfate caused a slow increase in loaf volume which reached no maximum up to 160×10^{-6} moles/100 g. flour. Figure 2 illustrates the antagonistic action between oxidizing and reducing agents upon loaf volume. Cysteine produced a slow but steady decrease in the volume of loaves treated with an optimum amount of bromate, while glutathione caused an increase in the volume of loaves treated with an excess amount of bromate. The volume here reached a maximum at 30×10^{-6} moles

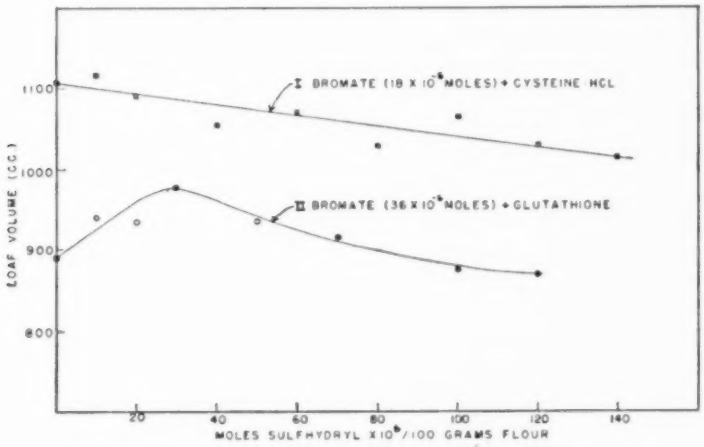

FIG. 2. Loaf volume from constant bromate and varying sulfhydryl treatment.

GSH/100 g. flour. The reversibility of the effect of excess bromate however was not complete. The maximum loaf volume 980 cc. was not the same as that for the optimum amount of bromate in Fig. 1, 1,050 cc. With a valence change of six for bromate, the number of equivalents of excess bromate for the second case was 108×10^{-6}, yet glutathione increased loaf volume up to an amount of only 30×10^{-6} equivalents. This seems to indicate that glutathione may react in some manner other than with equivalent quantities of bromate.

The lack of response to persulfate shown in Fig. 3 is surprising when compared with those for iodosobenzoate and bromate. The contrast in response to cysteine and glutathione points to some additional factor other than sulfhydryl content being active. Again iodoso-

benzoate is shown to be a more powerful improver than potassium bromate on an equivalent basis.

The antagonistic action of oxidizing and reducing agents on loaf volume of a high grade flour is shown in Fig. 4. Bromate produced a maximum recovery in loaf volume at a concentration of 14×10^{-6} moles/100 g. flour, while 70×10^{-6} moles of persulfate were required. In both cases the required amount of oxidizing agent for maximum loaf volume was greater than the amount of reducing agent added plus that amount of oxidant which gave maximum loaf volume in Fig. 3.

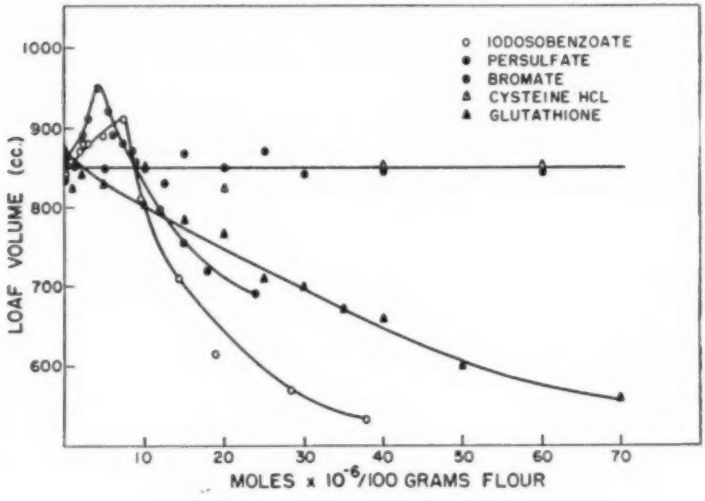

FIG. 3. Baking response of a top patent flour.

From Fig. 1, it is noted that cysteine and glutathione produce essentially the same effects upon the loaf volume of a clear flour. This however is not true for the top patent flour (Fig. 3). It is impossible to explain these differing results on the basis of a simple oxidation-reduction relationship. Some additional factor must be involved, and specificity of reaction and/or ease of adsorption of the reagent upon the flour are possibilities.

Discussion

In contrast to the conclusions of McDonald (12) that the iodosobenzoate titration method for sulfhydryl determinations is unreliable when applied to gluten dispersions, the results reported here indicate that when applied to flour systems other than gluten dispresions the method is quite precise. The concentration of reagents used in this work did not cause any significant flocculation of protein. The starch

endpoint in some cases was prone to fade, a result of protein-iodine reaction. In the results reported, all titrations were made to the first color which remained 30 seconds.

Many determinations were performed on gluten washings and protein-free washings. The results show about 70–90% of the sulfhydrly content of gluten washings remains in the filtrate following ammonium sulfate precipitation. In addition, evidence indicates that approximately one-third of the total sulfhydryl content of a flour is removed by the A.A.C.C. gluten washing procedure. From these results it is concluded that the bulk of the water-soluble compound containing this group is of relatively small molecular weight.

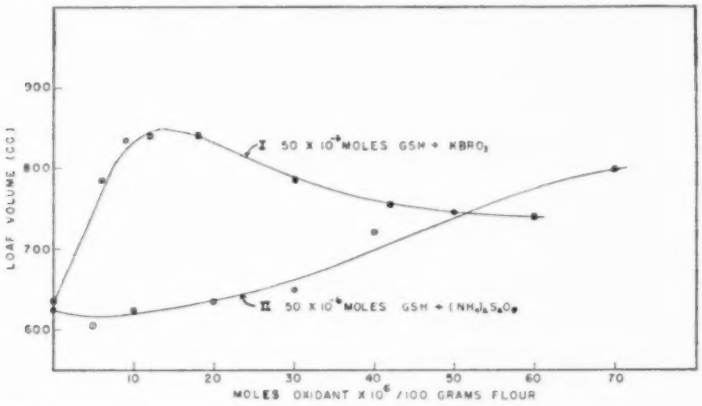

FIG. 4. Loaf volume from flour treated with constant excess glutathione and varying oxidants.

The lack of reaction of bromate and persulfate with these sulfhydryl groups in those experiments designed to test the effect of added oxidizing agents at room temperature is of particular interest.

Hlynka (8) has reported that no decrease in added bromate occurs in flour-water doughs during a three-hour period. This observation is in line with the results of the present study and show that in nonfermenting doughs the conditions are not optimum for oxidation of sulfhydryl groups. At the higher temperature of the oven the reaction may occur more rapidly. Hlynka found, however, that yeast-doughs bring about a decrease in bromate content during fermentation. Auerbach (1) reports loss of persulfate in flour-water doughs. With this result in mind it would appear that in yeast-doughs flour improvers must react with some groups other than the sulfhydryl groups which occur in the flour proteins, as determined with iodosobenzoate, during the stages of dough makeup and fermentation.

That the nature of the sulfhydryl compounds is the factor causing lack of reaction with oxidizing agents is shown by the disappearance of glutathione when this reagent is added to gluten washings. Additional experiments, not reported here, have shown that glutathione is oxidized by persulfate in aqueous solutions. Cysteine has also been shown to be the product of reaction of cysteine hydrochloride and potassium persulfate. No satisfactory explanation of the effect which the nature of the sulfhydryl group may have upon the reactivity can be given.

The results of the baking experiments show the diverse effects which certain chemical reagents have on the baking characteristics of wheat flour. All oxidizing agents did not produce equivalent quantitative responses. Their effectiveness decreased in the order: iodosobenzoate, bromate, iodate and persulfate. The non-uniform response of any one flour to these oxidants thereby suggests that certain unknown specific factors, other than oxidation of sulfhydryl groups, are operating.

The well-known fact that lower grades of flour require large amounts of improver is borne out by the following data from Figs. 1 and 3.

Flour	*Improver*	*Amount of Improver (equiv. × 10^{-6}/ 100 g. flour)*
top patent	*o*-iodosobenzoate	0.14
first clear		0.76
top patent	bromate	0.24
first clear		1.08
top patent	persulfate	no maximum
first clear		2.80

The varying requirements of any one flour for different improvers is a point which is rather difficult to explain. The various dosages of improving agents required for maximum loaf volume are generally interpreted as being related to the different rates at which they oxidize sulfhydryl groups. Since very little clear-cut data are available on the rates of oxidation of sulfhydryl groups by various flour improvers, confirmation of this explanation is not possible. It is true, however, that iodosobenzoate is the most effective improver used here, and it is known to oxidize sulfhydryl groups almost instantaneously. Variations in the specificity for adsorption of the flour improvers, especially if their action is in part associated with enzyme inhibition, would interfere with any relation between improver efficiency and the rates of oxidation.

Acknowledgements

Thanks are due to Mr. R. E. Teed for the baking results and to the Robin Hood Company, Saskatoon, Sask. for the commercial flour samples.

Literature Cited

1. AUERBACH, M. E., ECKERT, H. W., and ANGELL, ELEANOR. The determination of persulfate in flour and dough. Cereal Chem. **26:** 490–6 (1949).
2. BAKER, J. C., PARKER, H. K., and MIZE, M. D. The action of oxidizing agents on sulfhydryl compounds in dough. Cereal Chem. **21:** 97–107 (1944).
3. BALLS, A. K., and HALE, W. S. Cereal Chem. **13:** 55–56 (1936).
4. Cereal Laboratory Methods. 5th ed. Amer. Assn. Cer. Chem., St. Paul, Minn. (1947).
5. DOTY, J. M., and SHERWOOD, R. C. Experiments with ammonium persulfate as a flour maturing agent. Cereal Chem. **27:** 409–414 (1950).
6. HELLERMAN, L., CHINARD, F. P., and RAMSDELL, PAULINE A. *o*-Iodosobenzoic acid, a reagent for the estimation of cysteine, glutathione, and the substituent sulfhydryl groups of certain proteins. J. Am. Chem. Soc. **63:** 2551–3 (1941).
7. HLYNKA, I. Effect of bisulfite, acetaldehyde, and similar reagents on the physical properties of dough and gluten. Cereal Chem. **26:** 307–16 (1949).
8. HLYNKA, I. (personal communication) (Paper 18, 36th Annual A.A.C.C. Meeting 1951).
9. HLYNKA, I., and BASS, E. J. Reaction of dough and gluten with glucose. Cereal Chem. **26:** 513–518 (1949).
10. HLYNKA, I., and ANDERSON, J. A. Further studies on the reaction of wheat proteins with reducing carbohydrates. Cereal Chem. **28:** 136–144 (1951).
11. JORGENSON, H. Further investigations into the nature of the action of bromate and ascorbic acid on the baking strength of wheat flour. Cereal Chem. **16:** 51–60 (1939).
12. MCDONALD, D. J. Studies on the nature of wheat gluten. April 1948. Master's Thesis, University of Saskatchewan.
13. MYERS, H. S., and WORKING, E. B. The sulfhydryl groups of wheat flour. Cereal Chem. **21:** 32–37 (1944).
14. NORDIN, P., and SPENCER, E. Y. Determination of sulfhydryl groups in flour by means of *o*-iodosobenzoate and radioactive iodine. Cereal Chem. **29:** 29–39 (1952).
15. SULLIVAN, B. The mechanism of the oxidation and reduction of flour. Supplement to Cereal Chem. **24:** 16–31 (1948).
16. SULLIVAN, B., HOWE, M., SCHMALZ, F. D., and ASTLEFORD, G. R. The action of oxidizing and reducing agents on flour. Cereal Chem. **17:** 507–528 (1949).

ENZYMES THAT DEGRADE BARLEY GUMS. I. ISOLATION FROM A BACTERIAL SOURCE [1]

E. J. Bass, W. O. S. Meredith, and J. A. Anderson

ABSTRACT

An enzyme system that reduces viscosity and increases reducing power of aqueous barley gum solutions has been isolated from a commercial bacterial alpha-amylase preparation. The liberation of reducing groups is believed to be associated with the enzyme responsible for viscosity reduction. It is therefore suggested that the bacterial gum-degrading enzymes are a group of endo-glycosidases. The initial reaction between this enzyme system and its substrate follows zero order kinetics. The enzyme system can be separated from alpha-amylase by chromatography on activated alumina with 0.005 *M* phosphate buffer (pH 6.5) as eluting agent. In dilute solution the enzyme is inactivated by heating at 70°C. for 15 minutes. Extracts of green malt contain a similar endo-glycosidase system.

Studies of wort viscosity as a factor in malt quality (5) led to isolation from wort of a carbohydrate complex that forms viscous aqueous solutions. The complex, called "wort gum," contains about 3% nitrogen. A similar gum was extracted from barley flour (2). Both gums were degraded by a commercial bacterial alpha-amylase preparation that decreased viscosity and increased reducing power of aqueous gum solutions. Gum-degrading activity was essentially unchanged when alpha-amylase was inactivated by the procedure of Olson, Burkhart, and Dickson (7), but activity was negligible when enzyme solutions were first heated at 70°C. for 15 minutes. Extracts of green malt showed a similar gum-degrading activity. It thus appears that both the commercial enzyme preparation and green malt contain a carbohydrase system, other than the amylase system, of significance in the malting process. The enzyme system has been tentatively named X-enzyme.

The object of this research is to study the properties of barley and malt enzymes that degrade barley gums, and the role these enzymes play in the malting process. This paper presents results of preliminary studies on gum-degrading enzymes of the bacterial preparation and green malt. The bacterial preparation was investigated first, as this source is rich in X-enzyme, but appears to contain no beta-amylase

[1] Manuscript received April 10, 1952. Paper No. 118 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, and No. 299 of the Associate Committee on Grain Research (Canada). Presented at the Annual Meeting of the American Society of Brewing Chemists, Toronto, May, 1952.

and, presumably, few other complex materials, such as occur in malt extracts. A viscosimetric procedure for measuring the activity of bacterial X-enzyme and a chromatographic technique for purifying the enzyme system were developed. Attention was then directed to isolation of gum-degrading enzymes of green malt. Indications are that procedures for kinetics studies and purification of bacterial X-enzyme may be applied to malt X-enzyme.

Materials

Substrate. The principal substrate was a slightly modified raw endosperm gum, one of the barley gums reported in a previous paper (6). A 1% aqueous mixture of raw endosperm gum is heated under reflux on a boiling water bath for two hours with occasional stirring. The mixture is then cooled to room temperature and centrifuged. The supernatant is stirred into three volumes of 95% ethyl alcohol, and the precipitate is collected then washed successively with alcohol, acetone, and ether. The yield of modified gum is about 50% of the raw gum. The modified gum contains 0.7% nitrogen whereas raw endosperm gum contains about 3%. Aqueous solutions of modified gum are somewhat less viscous but far more stable than solutions of raw endosperm gum. One per cent solutions of modified gum have relative viscosities of 5 to 7 centipoises, and reduction in viscosity after 3 hours at room temperature is about 3%.

Substrate solutions were prepared by mixing gum with distilled water in the proportion of one gram of gum to 100 ml. of water. The mixture is stirred mechanically at low speed for half an hour, and filtered by suction. The filtrate is used as soon as possible to minimize effects of instability. Solutions prepared in this way are referred to as "1% solutions," but dry matter rarely exceeds 0.8%.

Bacterial X-enzyme. The source of bacterial X-enzyme was Wallerstein's "Alpha Amylase Special for Analytical Purposes." This is a preparation derived from a special strain of bacteria of the *B. subtilis* group.

Malt X-Enzyme. Malt X-enzyme was extracted from commercial 6-day malts provided by the Canada Malting Company.

Studies of Bacterial X-Enzyme

Methods. Enzyme-substrate reactions were studied by measuring changes in viscosity and reducing power at 30°C. of reaction mixtures containing 90 ml. of a 1% solution of modified gum and 10 ml. of a 5 mg. per cent solution of the bacterial preparation. Blanks contained 90 ml. of substrate and 10 ml. of the same enzyme solution heated at

70°C. for 15 minutes. Two drops of toluene were added to each reaction mixture and blank to inhibit bacteria. When reducing power of reaction mixtures had reached a maximum value, 15 ml. portions of each were concentrated to about 1 ml. by vacuum distillation. These concentrates were examined for sugars by descending paper chromatography. Ten drops of concentrate, containing at least 2 mg. of gum, were chromatographed. Ethyl acetate, pyridine, and water (5:2:5) were used as the solvent and aniline phosphate as the spray reagent. By this procedure a drop of solution containing 0.1 mg. of monosaccharide gives a distinct spot on the chromatogram; hence the limit of detection is about 0.01 mg.

For viscosity measurements, 5 ml. aliquots of reaction mixtures were pipetted into fast-flowing Ostwald viscosimeters (15 to 25 seconds for water at 30°C.) in fixed positions in a water bath maintained at 30°C. (±0.05°C.). Reactions were allowed to proceed in the viscosimeters as there was no evidence of shear. The time of a given reading was measured from the time substrate and enzyme were mixed and was corrected for time of flow in the viscosimeter. Curves of specific viscosity vs. time for blanks were linear; hence specific viscosity at zero time was obtained by extrapolation. Data were plotted as net decrease in specific viscosity vs. time.

Reducing power was determined by a method similar to the standard ferricyanide procedure for reducing substances (1). Five ml. aliquots were used, and ferricyanide and thiosulfate reagents were 0.05 normal. Data were plotted as net volume of ferricyanide reduced per aliquot vs. time.

Results. Figure 1 and Fig. 2 show typical curves of changes in viscosity and reducing power. Viscosity reduction appears to be more characteristic of the initial reaction than increase in reducing power, as specific viscosity decreases to a minimum value in about 2 hours, whereas maximum reducing power occurs after 5 days. Paper chromatograms showed no trace of mono- or di-saccharides; hence the degree of hydrolysis to simple sugars must be considerably less than 5%.

These preliminary results may be explained by postulating that X-enzyme is an endo-glycosidase system. Endo-glycosidic ruptures satisfactorily account for a rapid decrease in viscosity accompanied by a slower increase in reducing power, because viscosity may be profoundly affected by, say, a 50% reduction in molecular size, whereas the accompanying reducing power increase would be small. Hence the major drop in viscosity occurs rapidly, but reducing power may continue to increase for a considerably longer time. Moreover, the end products of endo-glycosidase action are presumably oligosaccharides

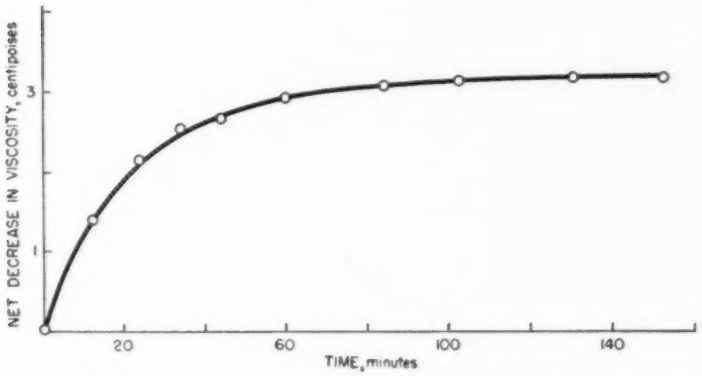

FIG. 1. Effect of bacterial enzymes on specific viscosity of a 1% solution of modified gum.

and polysaccharide fragments, with very little likelihood of mono- or di-saccharides.

Reaction Rates. Changes in specific viscosity are better suited to studies of X-enzyme kinetics than changes in reducing power, as viscosity reduction is a more accurate measure of initial rate. Therefore reaction data were studied by plotting η_o/η_t against time for reaction mixture and blank (where η_o is specific viscosity at zero reaction time, and η_t is specific viscosity at time, t). Reaction curves for X-enzyme are obtained by subtracting curves for blanks from curves for reaction

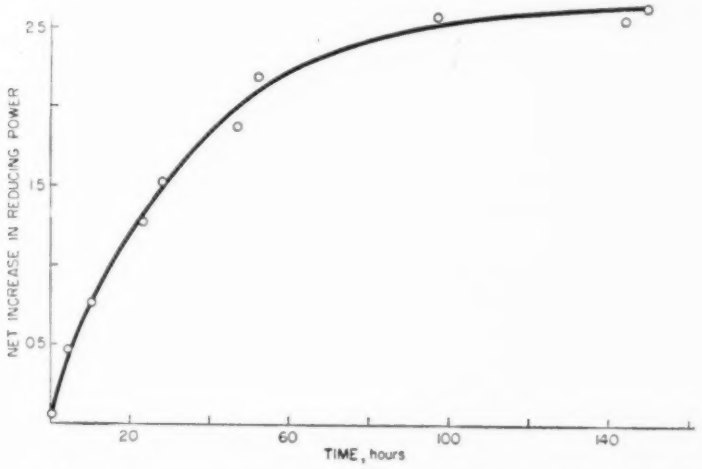

FIG. 2. Effect of bacterial enzymes on reducing power of a 1% solution of modified gum.

mixtures. Figure 3 shows typical reaction curves for reaction mixtures containing 9 ml. of a 1% solution of modified gum and 1 ml. of a 7.5 mg.% solution of bacterial preparation. The curve for X-enzyme is linear in initial stages of the reaction, and the slope of the linear

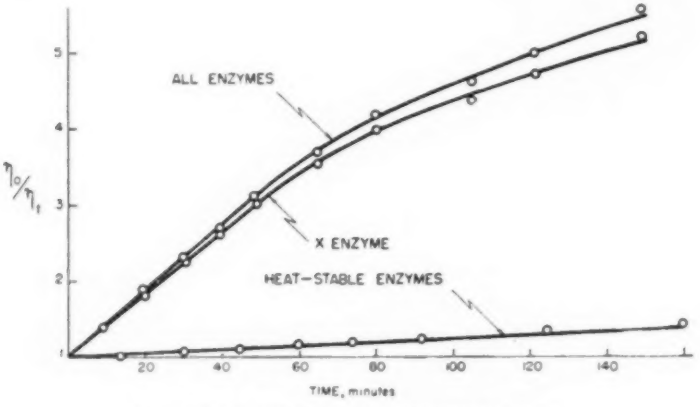

FIG. 3. Typical reaction curves for the bacterial enzymes.

portion is a measure of X activity. Figure 4 shows that there is a linear relation between slope and enzyme concentration.

A detailed study of enzyme kinetics was not feasible at this time as modified gum is not a completely satisfactory substrate. Concentrated solutions of the gum (about 0.8 g. per 100 ml.) have initial

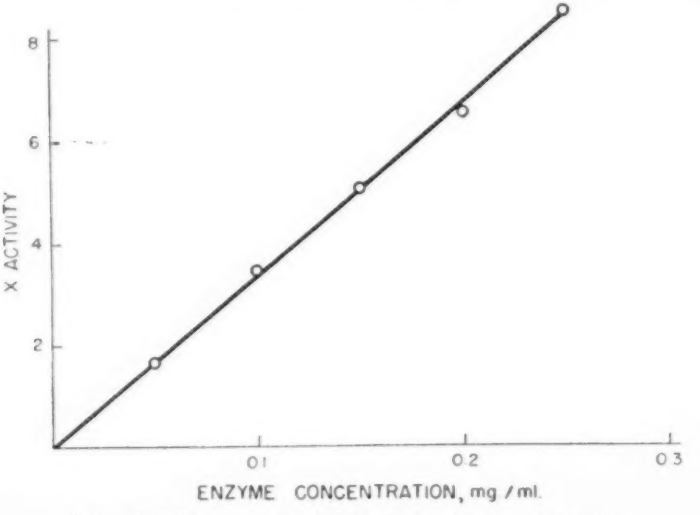

FIG. 4. Effect of concentration of the bacterial preparation on X-activity.

viscosities of 5 to 7 centipoises; hence the effect of substrate concentration on reaction rate can be studied only over a very narrow range of substrate concentration. Attention was therefore directed towards purifying the bacterial enzyme by chromatography while new gums were being prepared and tested for viscosity, stability, and specificity towards X-enzyme.

Chromatographic Purification of Bacterial X-Enzyme

Methods. An adsorption column of ¾-in. inside diameter was packed to a height of 3 in. with a paste of activated alumina[2] in distilled water, then washed with about 100 ml. of distilled water. One ml. of a 1% solution of the bacterial preparation was passed into the column, followed by another 50 ml. of distilled water. The column was then developed with phosphate buffer, and effluents were collected in 10 ml. portions. Solutions of the bacterial preparation showed an absorption maximum at 265 mμ, and effluents high in X activity also absorbed strongly at this wave length. Hence effluents with optical densities of 0.1 or higher at 265 mμ, measured in a Beckman spectrophotometer with a silica absorption cell of 1 cm. thickness, were combined and tested quantitatively for X activity and alpha-amylase activity. These quantitative measurements of activity were then converted to per cent enzyme recoveries, as outlined next. Optimum conditions for separating X-enzyme from alpha-amylase could then be determined by observing the effect of varying conditions on per cent enzyme recovery.

X activity of the combined effluents was determined by the slope of the linear portion of the reaction curve obtained with modified gum as substrate. The concentration of X-enzyme in the effluent was then estimated in terms of the bacterial preparation by referring to a standard curve such as Fig. 4. For example, an effluent with X activity of 1.7 should have the same concentration of X-enzyme as a solution containing 0.05 mg. of bacterial preparation per ml. The total X-enzyme content of effluent, and hence the per cent recovery, may then be calculated if the weight of bacterial preparation chromatographed and total volume of effluent are known.

Alpha-amylase activity was determined by a method similar to the standard starch-iodine colorimetric method (1) except that colors were estimated visually. Alpha-amylase activity is inversely proportional to the time required to develop a standard color, and is assumed to be zero if there is no change in the blue starch-iodine color after an incubation period of at least 48 hours. Per cent enzyme recovery was calculated as before from concentration in terms of bacterial preparation.

[2] Brickman's "alumina for chromatography," activated by the method of Dupont, Dulou, and Vilkas (3).

Buffer Concentration. Ten milligrams of the bacterial preparation (1 ml. of a 1% solution) were adsorbed on the column and developed with phosphate buffers of various concentrations from 0.002 *M* to 0.01 *M*. The pH of all buffer solutions was arbitrarily adjusted to 8.4, and a fresh column was prepared for each fractionation. Figure 5 shows that X-enzyme is eluted preferentially at the lower concentrations. As buffer concentration increases, recovery of X-enzyme increases. When the buffer is about 0.006 *M* a trace of alpha-amylase

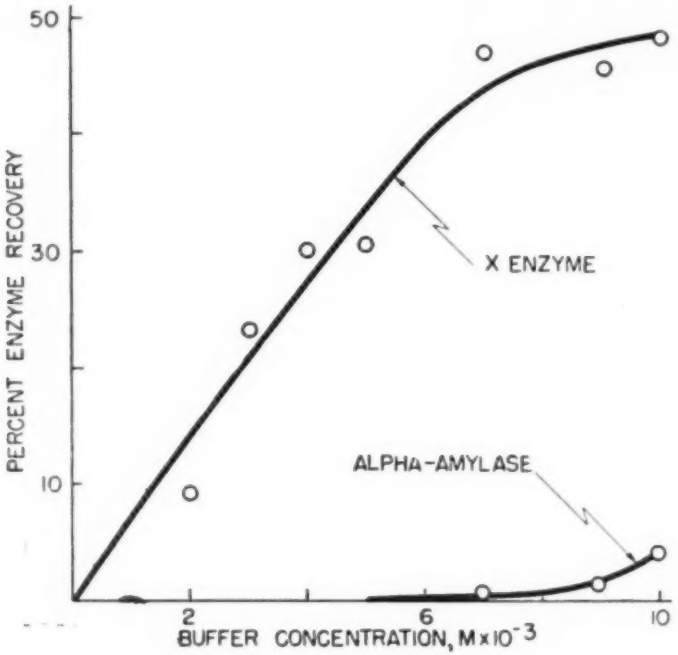

FIG. 5. Effect of phosphate buffer concentration (pH 8.4) on recovery of bacterial X-enzyme and alpha-amylase.

appears in the effluent containing X-enzyme, and recoveries of both enzymes increase until the buffer concentration is 0.009 *M*. Recovery of alpha-amylase continues to increase as buffer concentration is increased, but recovery of X-enzyme appears to level off at about 47%. The optimum buffer concentration is therefore 0.005 *M*.

Buffer pH. Several 0.005 *M* phosphate buffer solutions varying from pH 3.5 to pH 9.0 were used to fractionate 10 mg. samples of the bacterial preparation. A fresh column was prepared for each developer, and effluents containing X-enzyme were adjusted to pH 6.0 with

a minimum volume of hydrochloric acid or sodium hydroxide. All effluents showed no trace of alpha-amylase activity. Figure 6 shows that recovery of X-enzyme is very low at extreme pH's and high at pH 6.5 to 8.0. Maximum recovery occurs at pH 6.5. Optimum buffer pH is therefore 6.5.

Capacity of Column. Capacity of the column was determined by developing 100 mg. and 400 mg. of the bacterial preparation with 0.005 *M* phosphate buffer of pH 6.5. Alpha-amylase recoveries were zero, and 0.05% respectively. Hence the column can accommodate between 100 mg. and 400 mg., and 100 mg. appears to be a safe maximum.

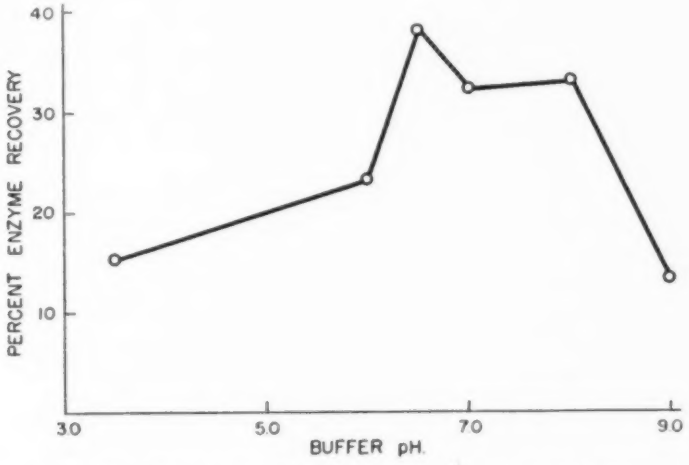

FIG. 6. Effect of phosphate buffer pH (0.005 *M*) on recovery of bacterial X-enzyme.

Heat Sensitivity. Chromatographic effluents showed no X activity after heating at 70°C. for 15 minutes. Hence purified X-enzyme is completely inactivated by heating at 70°C. for 15 minutes. Presumably, the heat-stable enzymes of the crude preparation that are removed by chromatography are either minor constituents of the gum-degrading enzyme system or other enzymes acting on impurities in the substrate.

Gum-Degrading Enzymes of Green Malt

Preliminary experiments with extracts of green malt were designed to see if malt X-enzyme could be studied by the methods developed for studying bacterial X-enzyme.

Extraction and Precipitation. Fifty grams of green malt were macerated with 200 ml. of distilled water in a Waring blendor for 2 minutes.

The mixture was centrifuged and the supernatant stored overnight at 7°C. It was then clarified by centrifugation and filtration, and saturated ammonium sulfate solution was added in graded amounts until the filtrate was half saturated with ammonium sulfate. After each addition the precipitate was removed by centrifugation and filtration, washed with half saturated ammonium sulfate, acetone, then air-dried. The remaining liquor was then saturated with ammonium sulfate, and the precipitate was removed, washed with saturated ammonium sulfate solution, acetone, then air-dried. Liquors and acetone-dried precipitates were tested for X-enzyme and alpha-amylase activities.

Properties of Isolates. The clarified malt extract showed considerable viscosity reducing activity. Curves of η_o/η_t vs. time were similar to reaction curves for bacterial X-enzyme, with an initial linear period followed by a period of gradually diminishing slope. Heating at 70°C. for 15 minutes destroyed most of the viscosity reducing activity. Therefore the action of malt X-enzyme appears similar to that of bacterial X-enzyme, and kinetics of both enzyme systems may be studied by similar procedures.

X activity of the acetone-dried precipitates increased slowly as ammonium sulfate concentration increased, but most of the enzyme was precipitated at concentrations higher than half saturation. Alpha-amylase activity increased more rapidly with increased ammonium sulfate concentration, and the bulk of this enzyme was precipitated by the time the malt extract was half saturated. Hence a partial separation of the two enzymes may be effected by precipitation from half saturated and saturated ammonium sulfate solutions.

As the final liquor showed no X activity, it appears that ammonium sulfate precipitates X-enzyme completely. However, enzyme yields were considerably lower than those calculated from activities of the liquors. Improved techniques will no doubt result in higher yields, but it may prove interesting to study the factors governing X-enzyme recovery.

Further studies of malt X-enzyme are in progress.

Discussion

Some conclusions about the mechanism of the reaction between bacterial X-enzyme and barley gums may be drawn from the preliminary results. The shape of reaction curves, such as Fig. 3, shows that there is an initial zero order reaction, with a constant reaction rate, followed by a period of gradually diminishing rate. This suggests that substrate molecules are degraded in steps of progressively increasing activation energies, and therefore progressively decreasing reaction rates. Thus, if a single molecule of substrate is considered, the first

endo scission is associated with a minimum characteristic activation energy. The two fragments are then subject to further degradation, but at higher activation energies. As molecular size decreases, activation energy increases and reaction rate decreases. When molecular size has been reduced below a critical size, endo action ceases. The end products of the reaction are presumably oligosaccharides and polysaccharide fragments that are resistant to endo action.

Due to the complexity of the reaction, only the initial zero order reaction period, corresponding to primary breakdown of substrate, is significant in studies of X-enzyme kinetics. In all probability, the total reaction rate at more advanced stages of the reaction is the sum of a variety of different rates depending on the relative amounts of undegraded gum and degradation products, and the nature of these substrates. Theoretically, the initial period may be studied by measuring either viscosity or reducing power. However, reducing power at early stages is small and may therefore be subject to considerable experimental error, particularly if the reacting system is contaminated with small amounts of starch and amylases. Hence the viscosimetric method is preferable for studies of bacterial X-enzyme.

As reaction curves for green malt enzymes are similar to curves for bacterial X-enzyme, the mechanism of malt X-enzyme action appears to be similar to that of the bacterial enzyme system. The argument in favor of a viscosimetric method for studying malt X-enzyme is strengthened by evidence, in the literature, that green malt may contain another gum-degrading enzyme system whose action also increases reducing power. For example, Lüers and Volkamer (4) identified xylose as a product of the degradation of a barley xylan by "cytase" of green malt. More recently, Preece, Ashworth, and Hunter (8) suggested that there are two aspects of cytase action: (a) a cytoclastic action, agreeing in principle with the concept of an endo-glycosidase system; (b) a cytolytic action characterized by a prolonged liberation of reducing groups. Hence reducing power contributed by malt X-enzyme may be obscured by the action of the second enzyme system. It is tempting to speculate that the second enzyme system may be an exo-glycosidase system, as such an enzyme could have little or no effect on viscosity in early stages, and would be characterized by a more or less constant activation energy throughout its action. The two gum-degrading enzyme systems of green malt could then be studied concurrently, endo-glycosidases by viscosity measurements in early stages of the reaction, exo-glycosidases by reducing power measurements in more advanced stages when endo action is virtually complete. It is significant that Preece's data may be satisfactorily explained by pos-

tulating the existence, in green malt, of systems of endo- and exo-glycosidases, but there are as yet few data to support this view.

Studies on the mode of action of gum-degrading enzymes are hampered by the lack of chemical data characterizing either the substrate attacked or the products formed. Some progress has been made in fractionating barley gum, and it now appears that it is a mixture of three or more polysaccharides differing in composition and structure.[3] If more than one of these fractions is degraded by X-enzyme, then X-enzyme may not be a single enzyme. Moreover, the role of nitrogen in barley gums is not yet clear, and it may be that the initial rapid reduction in viscosity of aqueous solutions of barley gum is caused by an enzyme system that removes cementing protein. However, recent work fails to confirm this possibility, since the initial rapid action occurs with barley gum containing as little as 0.08% nitrogen. Nevertheless, such an enzyme system may play an important part in releasing water-soluble polysaccharides bound by protein in hemicellulose materials, and may therefore be an important factor in malt modification.

Literature Cited

1. American Society of Brewing Chemists. Methods of analysis. Fifth Edition (1949).
2. Anderson, J. A., and Aitken, T. R. Twenty-third annual report (1949), Grain Research Laboratory, Board of Grain Commissioners for Canada, 1950.
3. Dupont, G., Dulou, R., and Vilkas, M. Préparation d'alumine pour chromatographie. Bull. Soc. Chim. (France). **7–8:** 785–786 (1948).
4. Lüers, H., and Volkamer, W. Das Hemizellulose spaltende Enzym (Zytase) des Malzes. Wochschr. Brau. **45:** 83–87, 95–99 (1928).
5. Meredith, W. O. S. Studies on wort turbidity, viscosity and degree of attenuation in barley malt. Thesis, University of Manitoba (1949).
6. Meredith, W. O. S., Bass, E. J., and Anderson, J. A. Some characteristics of barley, malt and wort gums. Cereal Chem. **28:** 177–188 (1951).
7. Olson, W. J., Burkhart, B. A., and Dickson, A. D. Inactivation of alpha- and beta-amylase in aqueous malt extracts. Cereal Chem. **20:** 126–138 (1943).
8. Preece, I. A., Ashworth, A. S., and (in part) Hunter, A. D. Cytolysis in germinating barley. II. Preliminary study of enzyme relationships. J. Inst. Brewing. **56:** 40–47 (1950).

[3] Personal communication from Dr. F. Smith, Department of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.

EXPOSED GERM IN WHEAT. A FACTOR IN WASHER FOAM PRODUCTION[1]

C. R. JONES and G. J. BAKER[2]

ABSTRACT

Imported wheats contain on the average about 25 per cent of grains whose germs are totally or partly exposed as a result of commercial handling of the dry grain. The exposure may also be produced by scouring processes. Parcels of English wheat show relatively intact bran coverings, because the grain is handled at relatively high moisture contents.

The tendency of wheat to produce a stable foam during short periods of agitation with water, as in wheat washing, is dependent on the presence of exposed germ, and is increased by a factor believed to be loosening of the germ caused by scouring or by commercial handling of dry grain.

Many commercial wheats arriving at London docks contain large percentages of grains whose germs are more or less exposed through partial or total removal of the bran coats over the germ. A particular practical consequence, which first drew our attention to this feature of wheat, is that of increasing foam production in commercial wheat washing with a type of washer in which the wheat is conveyed, through a trough of water, into a whizzer whose base runs below water level. Foaming of the water returning to the tank from the whizzer is controlled by water sprays. If these are not fully efficient the ease of control varies greatly from one wheat to another.

It was found that samples of troublesome wheat could be readily distinguished from those of other wheat by a simple foaming test. This paper deals with the application of the test to a number of imported and English wheats and with the relation between foam production and the extent of germ exposure as influenced by such factors as moisture content, wheat type and severity of mechanical treatment.

Materials and Methods

Samples of wheat examined were in four groups:

Group 1—18 samples of Nos. 1 and 2 Manitoba Northern wheats were taken from the holds of steamers in London docks during the period November 1949—February 1950. Of U. S. wheats, ten samples of No. 1 Heavy Northern Spring, one of No. 1 and two of No. 2 Hard Winter, two of No. 2 Red Winter and one of Soft White were taken

[1] Manuscript received February 4, 1952.
[2] The Research Association of British Flour Millers, Cereals Research Station, St. Albans, England.

from boats at various English ports including London during the period November 1950–May 1951. All these samples were received by courtesy of the Grain Committees of the respective Port Areas.

Group 2—Samples of various types arriving by barge at London mills. The data in Table I relate to three samples of Australian, one each of Durum, Chilean, Plate (64 lb. Rosafe) and Polish and three of English received in this way.

Group 3—Thirteen samples of rick-stored (non-combine harvested) English wheat of 1950 crop as delivered to a country mill by farmers and grain merchants. Two samples of combine-harvested and dried English wheat received by courtesy of the same country millers.

Group 4—Samples of Manitoba wheat (Winnipeg Inspection Averages and Export Standard Samples) sent direct to St. Albans from Canada, by courtesy of the Board of Grain Commissioners for Canada. Samples of Marquis wheat, similarly sent in connection with the 1947 and 1949 Collaborative Varietal Tests. Samples of this group will be referred to as "mailed" samples to distinguish them in discussion from samples of wheat imported commercially.

Foaming Test. Not less than 5 g. of wheat is shaken in a stoppered tube with twice its weight of water at 20°C. for 20 seconds and the behavior of the foam then observed. Measurements of depth of foam in millimeters were made on the side of a tube of diameter 2.5 cm. after shaking either 5 or 10 g. of wheat, according to quantity available, with 10 or 20 ml. respectively, of water for 20 seconds.

Germ Exposure Test. Ordinarily the extent to which germs are uncovered can only be assessed by tedious examination of a number of grains under medium power magnification. The examination however is facilitated by immersing the grains in warm water for a short period, thereby causing the germs to swell.

FIG. 1. Germ exposure in commercial wheat. From left to right: (1) total, (2) partial, (3) pin-head, (4) none.

Fifty grains are soaked for 20 minutes in water at approx. 55°C. The water is then drained from the grains and these are tipped into a shallow glass dish placed on a black surface. The grains are just covered with water (this assists, optically, the detection of exposed germ) and examined with an ordinary magnifying glass. *Exposed* germs appear as bright white swellings clearly defined against the dark tint of the adjacent bran coats. Germs of individual grains will ordinarily be exposed to different extents. For convenience four degrees of exposure, illustrated in Fig. 1, may be recognized: (1) total, (2) partial, (3) pin-head, (4) none (bran intact around germ). Grains in each class may be placed in rows, counted and the results expressed as a percentage of the total number of grains taken. The examination takes only two or three minutes, and tests on 50 grains give reasonably reproducible results.

TABLE I

GERM EXPOSURE IN VARIOUS WHEATS

Group[1]	Wheat Type	Germ Exposure			
		Total	Partial	Pin-head	None
		%	%	%	%
1	Imported Manitoba				
	Range for 18 samples	0–4	4–56	17–53	17–67
	Mean for 18 samples	1	25	34	40
1	Imported American				
	No. 1 Heavy Northern Spring				
	Range for 10 samples	0–4	0–16	47–66	24–46
	Mean for 10 samples	1	7	58	34
	Nos. 1 and 2 Hard Winter	3	6	61	30
	No. 2 Red Winter	1	5	47	47
	Mixed Soft White	0	8	53	39
2	Imported Australian	3	77	11	9
2	Imported Durum[2]	4	74	13	9
2	Imported Rosafe (64 lb.)	3	24	55	18
2	Imported Polish	1	9	52	38
2	Imported Chilean	0	4	12	84
2	English at London mill	0	7	8	85
3	English at country mill				
	Combine-harvested dried	0	0	3	97
	Rick-stored red and white from farmers:				
	Range for 13 samples	0	0	0–8	92–100
	Mean for 13 samples	0	0	4	96
4	No. 1 Manitoba Northern Export Standard (1948 crop)	0	4	6	90

[1] Group, under "Materials and Methods," in which the samples fell.
[2] No. 3 Canadian Western Amber Durum.

Results and Discussion

Extent of Germ Exposure in Various Wheats. Table I shows the results of germ exposure tests on samples of various wheats. While

commercial English wheats contained little exposed germ, all imported wheats examined, with the exception of Chilean, contained large proportions. On the other hand, Manitoba wheat, in the form of Winnipeg Inspection Averages and Export Standard Samples, contains little exposed germ. Dr. J. A. Anderson,[3] in a private communication, suggested that the explanation of this difference lay in the different handling received by the wheats. Later he kindly sent data, reproduced here as Table II, supporting this explanation. The samples from railway shipments represent those taken by stabbing carlots at certain central points (e.g., Winnipeg), for primary inspection and grading, the carlots having been loaded at the country elevators (small silos) to which the Canadian farmers deliver their grain. Of the 50 such samples shown in Table II, 25 represented straight grades with moisture contents of 14.5% and under and 25 represented carlots of tough and damp grain of higher moisture contents. The grades represented in Table II were No. 3 Northern and higher.

TABLE II

GERM EXPOSURE IN CANADIAN WHEAT

(Data obtained by the Grain Research Laboratory, Winnipeg, using the method described in this paper)

Description of Sample	No. of Samples	Germ Exposure			
		Total	Slight	Pin-head	None
		%	%	%	%
Railway shipments, 1950–51:					
Tough and damp grades	25	0.5	1.2	8.5	89.8
Straight grades	25	3.4	2.2	8.0	86.4
Lakehead cargoes, 1950–51:					
Tough and damp grades	13	2.5	3.2	14.2	80.1
Straight grades	25	3.6	11.4	24.0	58.8
Lakehead cargoes, 1949–50:					
Straight grades	25	3.3	7.2	28.9	60.7

The carlots are unloaded at the terminal elevators and the wheat is then cleaned. It also receives considerable handling as a result of unloading and loading operations. The three lower rows of Table II relate to samples taken after these operations. While there is some germ exposure in straight grades entering terminal elevators, the amount of exposure is considerably increased by the handling in terminals. The results also suggest a marked effect of moisture content on the extent to which germ exposure is produced. The increase in germ exposure caused by handling, more particularly at the terminal elevators, is far less for the tough and damp grades than it is for straight

[3] Chief Chemist, Dominion Grain Research Laboratory, Winnipeg.

grades. The authors agree with Dr. Anderson that the relatively small amount of germ exposure shown by English wheat probably depends on the fact that it is normally handled at a much higher moisture content (say 17–18%) than most imported wheats. Conversely, while European millers value dryness in imported and domestic wheats, this may be a factor contributing to germ exposure.

Effect of Scouring of Grain on Germ Exposure. Table III shows results of germ exposure tests and of foaming tests on sub-samples of a Winnipeg Inspection Average Sample, (a) untreated and (b) after various numbers of successive passages through a laboratory scourer. Two successive scourings caused a marked decrease in percentage of unexposed germs but fully exposed germs began to appear only after several scourings. Tests in a mill indicated that a commercial-scale scourer produced similar results.

TABLE III

THE EFFECT OF SUCCESSIVE SCOURINGS OF "MAILED" MANITOBA WHEAT[1] ON GERM EXPOSURE AND FOAM PRODUCTION

Number of Successive Scourings	Germ Exposure:				Depth of Foam[2]
	Total	Partial	Pin-head	None	
	%	%	%	%	*mm.*
Nil (control)	—	8	8	84	1.5
2	—	14	38	48	4.0
4	—	16	58	26	5.5
8	4	48	42	6	7.0

[1] Winnipeg inspection average sample of No. 1 Manitoba Northern (1948–49).
[2] With 10 g. wheat in 20 ml. water.

The Relation of Foam Production to Germ Exposure. The figures of Table III for "mailed" wheat suggest that results of foaming tests follow those of germ exposure tests. With regard to commercial samples, most of the imported wheats of Table I produced foam to a depth in the range 4.5–6.0 mm. (with 10 g. wheat), but English wheats failed to give appreciable foam.

The indication, that germ exposure affects foam production, was confirmed by the following experiment. The germ of each grain in a 5 g. sub-sample of a troublesome wheat was covered with sealing-wax; in another 5 g. sub-sample the germs were left untouched, but a roughly equivalent amount of sealing-wax was put on the beard end of each grain. The appearance of these sub-samples after shaking with water in comparison with a sample of non-troublesome Manitoba is shown in Fig. 2. No foam at all was formed by the wheat with sealed germs, but the presence of the sealing-wax on the beard ends did not lessen the foaming caused by this wheat. In another experiment a sample of

wheat, more than 90% of the germs of which were unexposed, was found to produce almost no foam, but, after the germ of each grain in a 5 g. sample had been peeled, foam was produced on shaking. Evidently, during the few seconds' contact with water, a foam-producing substance is dissolved out of the germ in sufficient concentration to produce foam when the solution is shaken. This substance may be lipid material or possibly a soluble protein, whose rapid solution is prevented, or hindered, when the bran covering the germ is intact. It was noticed that water was almost colorless after shaking for 20 seconds with grains whose germs were unexposed, but distinctly yellow with wheat containing an appreciable amount of exposed germ.

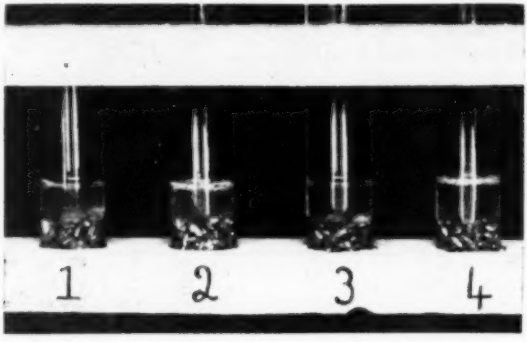

FIG. 2. Results of shaking Manitoba wheats with water for twenty seconds. 1. Non-troublesome wheat, germs untouched. 2. Troublesome wheat, germs untouched. 3. Troublesome wheat, germs covered with sealing wax. 4. Troublesome wheat, germs untouched, sealing wax on beard ends.

Pressure of other work has so far prevented investigation of this foam-producing substance but the following points concerning production of the foam have been ascertained:

(1) The optimum concentration corresponds to a 2:1 ratio of water to wheat.
(2) The optimum temperature is about 20°C. but small variations have little effect.
(3) The difference between wheats with exposed and unexposed germs is only shown fully with periods of contact with water shorter than about one minute. On prolonged contact some foaming occurs with grains whose germ coverings are intact.
(4) The extent of foaming is less with distilled water than with hard tap water. The presence of salts appears to assist solution of the responsible agent.

While the factor governing the foaming tendency is primarily that of germ exposure, the extent of foam production depends on two other factors connected with the wheat:

1. The method of production of the exposure whether by manual dissection or by a mechanical process such as scouring.
2. The type of wheat.

Effect of Method of Production of Germ Exposure. Table IV shows results of germ exposure and foaming tests on samples of commercially imported No. 2 Manitoba Northern and of Marquis wheat mailed direct from Winnipeg. The bottom two rows relate to sub-samples consisting (a) only of grains having pin-head exposure and (b) only of grains with completely exposed germs.

The grains of (a) were mostly picked out from the initial sample and were supplemented with specimens prepared by puncturing the bran covering the tip of the germ in sound grains. Those of (b) were prepared under a magnifier from sound grains present in the initial sample, by scraping away the bran covering the germ by means of a scalpel.

TABLE IV

EFFECT ON FOAMING OF INCREASE BY DISSECTION IN GERM EXPOSURE IN COMMERCIALLY IMPORTED AND IN "MAILED" CANADIAN WHEAT

	Imported Manitoba	Marquis ("Mailed")
Initial sample		
Germ exposure:		
Total %	10	Nil
Partial %	41	Nil
Pin-head %	36	15
None %	13	85
Dept of foam,[1] mm.	3.5	Negligible
Selected grains		
1. Pin-head only:		
Depth of foam,[1] mm.	3.5	Negligible
2. Total exposure only:		
Depth of foam,[1] mm.	3.5	1.0

[1] With 5 g. wheat, 10 ml. water.

Results resembling those for the commercial wheat shown in Table IV were obtained in other similar tests on imported Manitoba wheats and No. 1 Heavy Northern Spring wheat, while figures similar to those for Marquis were obtained with other "mailed" Canadian samples and also with English wheat.

It is apparent that with commercial wheat, in which much exposed germ is already present, foaming is not increased through manually increasing the percentage of exposed germs. With such wheat, grains showing only pin-head exposure foam as much as those with wholly exposed germs. With wheats containing, initially, little exposed germ the extent of foaming is increased by further germ exposure but, even with 100% exposure produced manually, it remains less than that with

commercial wheat. As shown in Table III, however, when such grain is treated several times in the laboratory scourer, its foam production is raised to that of commercial wheat of the same type.

This scouring treatment produces partial shattering of the bran coats over the main part of the grain. Grains of most commercially imported wheats also have this appearance, but the brans of "mailed" Canadian wheats are almost undamaged. The results in Table V show, however, that foam production is associated only with the germ.

These findings may be explained by supposing that, either in scouring or in the commercial handling of dry wheat, the germ becomes loosened mechanically so that soluble matter is more readily extracted from it by the water in the short period of shaking employed. It is well known that with some wheats, especially if dry, scouring may cause a proportion of the germs to become detached completely from their

TABLE V

FOAM PRODUCTION WITH "MAILED" MARQUIS WHEAT VARIOUSLY TREATED

Treatment of Grain		Depth of Foam[1]
Scouring	Special Treatment	
		mm.
None	Germs totally exposed manually	1.5
Six times	None	3
Six times	Germs totally exposed manually[2]	3
Six times	Germs, only, coated with sealing wax[2]	Nil
Six times	Beard ends, only, coated with sealing wax[2]	3

[1] With 5 g. in 10 ml.
[2] After scouring.

grains. An experiment in which embryos of all grains in a sub-sample were removed manually, leaving only the scutella fully exposed, did not indicate that scutellum has greater foaming power than the embryo (by which term we mean the assembly of radicle and plumule).

From this point of view, though germ exposure is a pre-requisite of foam production in the brief period of wheat washing, its extent does not directly determine the amount of foam produced by commercial wheats. However, like the general cracking of the bran, it is indicative of the degree to which the germ has been loosened by handling of the grain and this, in turn, affects the extent of foam production. At the same time, there is a point (not yet well-defined) beyond which additional damage in commercial wheats does not cause increased foam. Thus, a parcel of imported Manitoba wheat, which was unusually troublesome in commercial washing, gave the following percentage figures for germ exposure: total, 10; partial, 50; pin-head, 35; none, 5.

A sample was treated in the laboratory scourer until its content of totally and partially exposed germs had increased from 60 to 94%, but the depth of foam was not materially increased.

The Effect of Moisture Content and of Type of Wheat. The increase in germ exposure due to repeated laboratory scouring of "mailed" Manitoba wheat has been shown in Table III. Table VI shows the effect of similar scouring treatments (six successive passages through the laboratory scourer) on germ exposure and foam production of English wheat at its "natural" moisture content (19.1%) and of the same sample after gentle laboratory drying at about 30°C. to 13.4%. For comparison, figures are given for a mailed Marquis sample at 13.2%.

TABLE VI

THE EFFECT ON GERM EXPOSURE AND FOAM PRODUCTION OF SCOURING ENGLISH WHEAT AT HIGH AND LOW MOISTURE CONTENTS

Sample	Moisture Content	Germ Exposure				Depth of Foam
		Total	Partial	Pin-head	None	
	%	%	%	%	%	*mm.*
English unscoured	19.1	—	—	2	98	Nil
English scoured	19.1	—	—	24	76	V. slight
English unscoured	13.4	—	—	2	98	Nil
English scoured	13.4	10	40	38	12	1.5
Marquis unscoured	13.2	—	—	4	96	Nil
Marquis scoured	13.2	—	34	52	14	3

Thus the effect of scouring is quite slight on the English wheat at its natural moisture but after drying the effect is at least as great as on the Canadian wheat at the same moisture content. The drying treatment alone has no effect. These observations confirm the remarks made earlier in connection with Dr. Anderson's communication.

The dry-scoured English wheat was however found to produce less foam than the scoured Marquis. It has also been found that the depth of foam produced by imported Australian wheat is less than that given by Manitoba at corresponding degrees of germ exposure; moreover the foam from the English and Australian wheats subsides more quickly. Durum wheat and U. S. spring wheat appear to be in line with Manitoba wheat.

It is not known whether these differences are due to a lower content of foam-producing substance in germs of English and imported Australian wheats or to a difference in the degree of germ-looseness produced by handling of the grain.

THE REACTION OF FLOUR PROTEINS WITH SURFACE ACTIVE COMPOUNDS [1]

BETTY SULLIVAN [2]

ABSTRACT

Flour was treated with 0.1% to 1.0% of surface active compounds representing anionic, cationic, and nonionic types. At the normal pH of flour, detergents of the anionic type, as well as the sodium salts of the C_6 to C_{12} fatty acids, toughened the gluten and caused an extreme strengthening of the farinograph curve as measured by the consistency and amplitude. These changes were not reflected in the baking test to the degree that might be expected. Nonionic and cationic compounds showed little influence on the flour-water curve except at the 1.0% level where some weakness was evident. The mechanism of the interaction of flour proteins with anionic surface active compounds is not clearly understood.

The hot paste viscosity of flour, as measured by the amylograph, was increased by the surface active agents employed at levels much above 0.1%. Whether inhibition of alpha-amylase activity is involved needs further study.

Certain surface active compounds have been suggested for use in the tempering of wheat because such compounds decrease the time necessary for the penetration and distribution of water in the grain (6). The amount of such compounds occurring in the flour, at levels effective for tempering wheat, is so minute as to be exceedingly difficult to measure. As would be expected at the trace level present, they have no effect on the physical properties of dough or on the finished bread.

However, Swanson and Andrews (7, 8) showed that, when some surface active compounds, particularly Aerosol OT (sodium dioctylsulfosuccinate), are employed at levels of 0.06 to 0.24% based on the flour weight, they lengthened the time of development of the dough and decreased the rate of breakdown as indicated on the recording dough mixer curves. These authors considered that gluten is the main factor in the determination of the patterns of mixograms and stated that the most effective of the surface active agents tried in their experiments, Aerosol OT, had very little, if any, effect on starch. Among the group of detergents used by these authors, it was found that some had little, or no, effect on the curve patterns, yet they reduced the surface tension of water to about the same degree as those compounds which had marked effects. Swanson and Andrews (7) concluded that

[1] Manuscript received July 11, 1951. Presented at the Annual Meeting, May 1951.
[2] Russell-Miller Milling Company, Minneapolis, Minnesota.

the lengthening of the mixing time by surface active agents could not be explained by the common property of a decrease in surface tension and were at a loss to account for the differences in the effect of various compounds. They suggested that effective surface active agents caused a certain type of protein denaturation which allowed greater inter- or intramolecular penetration of water.

In recent years, the nature of the forces and groups involved in the reactions of proteins with other compounds has been studied by reacting relatively pure proteins, such as human or bovine serum albumin, beta lactoglobulin, and other proteins, with anionic and cationic detergents. Surface active compounds may be classified as anionic, cationic, or nonionic according to the sign of the charge of the hydrocarbon group conferring surface activity. Common examples of anionic detergents are alkyl aryl sulfonates, $CH_3CH_2(CH_2)_xCH_2C_6H_4SO_3^-Na^+$, alkyl sulfates, $CH_3CH_2(CH_2)_xCH_2OSO_3^-Na^+$, and fatty acid soaps such as $CH_3CH_2(CH_2)_xCH_2COO^-Na^+$. In many investigations, sodium dodecyl sulfate, $C_{12}H_{25}OSO_3^-Na^+$ (Duponol C), or the pure compound has been employed. These anionic compounds have a surface active or polar part of the molecule that carries a negative charge.

Cationic surface active agents, such as primary or secondary amine acid salts and quaternary ammonium compounds, carry a positive charge. They may be represented by a structure R R′ R″ R‴ N^+-X^- where X is a halogan and R is an aliphatic or cyclic group.

Nonionic surface active agents, such as polyether alcohols or polyoxyethylene sorbitan monostearate or mono-oleate, do not dissociate into positive or negative ions.

The action of detergents on proteins depends on experimental conditions such as detergent-protein mass ratio, pH and ionic strength of the medium, the sign and size of the charge of both reactants, and the molecular weight and structure of the detergents. Anionic and cationic detergents, in particular, produce many diverse effects on proteins, such as precipitation, protection from heat and other types of denaturation, enzyme and virus inactivation, and bactericidal action in the case of the cationic compounds. Anionic detergents are bound to proteins over a wide range of pH, but particularly at pH values lower than the isoelectric point of the protein. The negative group of the detergent combines with the positively charged groups of the protein. Cationic detergents also form complexes with some proteins, mainly by salt-like linkages between the positive groups of the detergent and the COO^- groups of the protein. Both types of detergents are believed by most investigators to produce the various complexes and effects by combination with proteins mainly through electrostatic forces, although loose Van der Waals' combinations have also been suggested. The

subject has been thoroughly discussed in reviews by Steinhardt (5), Putnam (4), and Lundgren (2).

It is the purpose of this paper to describe some experiments on the effect of anionic, cationic, and nonionic detergents on gluten quality and on farinograph curve characteristics as well as, incidentally, the influence of such compounds on the hot paste viscosity as measured on the amylograph. In a later paper, we hope to discuss the more theoretical aspects of the problem as they afford some insight into the structure of the gluten.

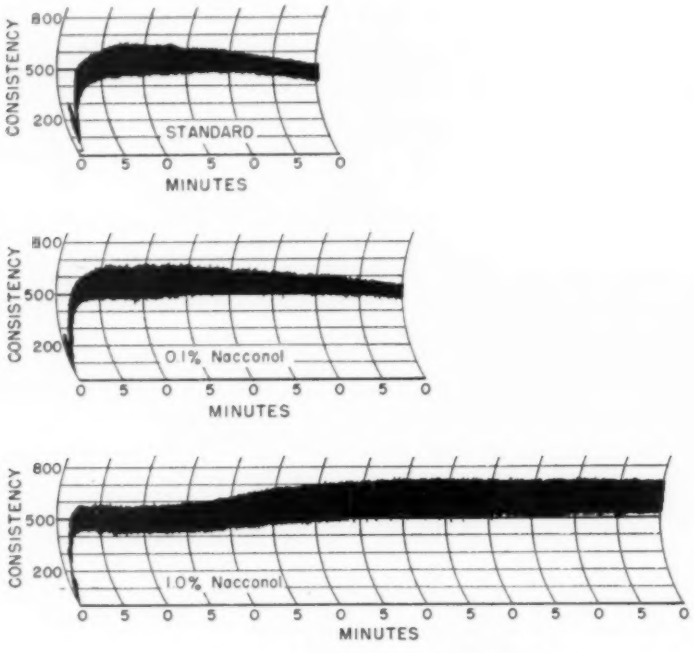

FIG. 1. Top, curve 1; Center, curve 9; Bottom, curve 10.

Materials and Methods

Except where otherwise noted, a straight grade, unbleached, spring wheat flour analyzing 14.20% protein and 0.48% ash was chosen for these experiments.

Glutens were washed in the usual manner from this flour and from the flour to which had been added three anionic detergents, Aerosol OT (dioctylsodiumsulfosuccinate), Nacconol NRSF (sodium alkyl aryl sulfonate), and Duponol C (sodium dodecyl sulfate); two nonionic

compounds, Tween 80 (polyoxyethylene sorbitan mono-oleate) and Triton X–100 (an alkyl aryl polyether alcohol); and two cationic compounds, Ammonyx T (cetyl dimethylbenzyl ammonium chloride) and Acidol 25-A (alkyl dimethyl amino hydroacetate). These compounds were used at 0.1%, 0.5%, and 1.0% levels of the content of the active compound based on the flour weight. An outstanding effect was observed only with anionic compounds, all of which toughened the gluten. This increased toughness began to be apparent at amounts of 0.1% and was very marked at 1.0% levels, the gluten acting like an old flour and remaining barely coherent.

Farinograph Curves. Farinograph curves were made using a constant dough weight (480 g.) and 550 units consistency for the control. The same weight of flour and the same absorption were used for curves when the detergent was added to the water. In such cases and with the amounts used, the pH of the flour-water-detergent mixture was 5.9 in all cases and no attempt was made to employ a range of pH levels. Again only the anionic compounds exhibited significant changes in the curve pattern. A large increase in the amplitude and apparent mixing time began to be noticeable, as in the washed gluten, at the 0.1% level and was pronounced at the 1.0% levels, as illustrated in Fig. 1 (Curves 1, 9, 10) and by the data given in Table I for curves 11 and 12. This corresponds to a detergent-protein ratio of 0.07. Nacconol (Fig. 1, curve 10) showed the greatest effect. Even at 70 minutes, the curve showed no breakdown and maintained the maximum consistency. Duponol C (Table I, curve 11) and Aerosol OT (Table I, curve 12) produced slightly less effect, the consistency decreasing slightly at 50 minutes and the amplitude becoming somewhat narrower at the same time. The Duponol curve reached a maximum slightly earlier than the other two compounds.

Since fatty acids are also anion-active compounds, it was of interest to compare the farinograph curves obtained with an homologous series of the sodium salts of fatty acids from C_6 (sodium caproate) to C_{12} (sodium laurate). A curve made with sodium oleate is included for comparative purposes. All the fatty acid soaps of the homologous series, at a 1.0% level, caused an increase in mixing time and in amplitude in proportion to the increase in molecular weight to C_{12}, as illustrated in Fig. 2, curve 5, and expressed by the data in Table I, curves 1 through 5. Sodium oleate (curve 6) caused a more marked change in the curve pattern than the lower saturated fatty acids. This curve showed the slow, irregular rise to maximum exhibited by an old flour. Sodium palmitate gave a curve very similar to the control and is not illustrated. None of the fatty acid soaps produced as striking changes in the curve patterns or in the feel of the washed gluten as the long-

TABLE I

DATA FROM FARINOGRAPH CURVES OF FLOUR WITH VARIOUS SURFACE-ACTIVE AGENTS

Curve	Time in Minutes															
	5		10		20		30		40		50		60		70	
	Consistency B.U.	Amplitude mm.	Consistency B.U.	Amplitude mm.	Consistency B.U.	Amplitude mm.	Consistency B.U.	Amplitude mm.	Consistency B.U.	Amplitude mm.	Consistency B.U.	Amplitude mm.	Consistency B.U.	Amplitude mm.	Consistency B.U.	Amplitude mm.
1. Standard	540	35	550	35	550	23	480	15	—	—	—	—	—	—	—	—
2. 1.0% Sodium caproate	490	35	500	38	520	28	480	19	430	16	—	—	—	—	—	—
3. 1.0% Sodium caprylate	500	35	530	38	580	33	540	23	500	18	470	17	450	15	—	—
4. 1.0% Sodium caprate	510	34	540	40	600	40	570	29	540	23	510	19	490	17	—	—
5. 1.0% Sodium laurate	510	33	500	34	540	37	560	37	530	26	490	22	480	17	—	—
6. 1.0% Sodium oleate	500	30	510	33	550	37	560	26	540	22	530	19	490	17	—	—
7. 1.0% Tween 80	560	40	590	35	560	23	490	17	—	—	—	—	—	—	—	—
8. 1.0% Triton X-100	540	33	580	30	530	20	480	17	—	—	—	—	—	—	—	—
9. 0.1% Nacconol	560	36	570	36	570	28	560	23	520	17	—	—	—	—	—	—
10. 1.0% Nacconol	500	30	500	30	540	38	590	46	620	48	620	47	630	43	620	38
11. 1.0% Duponol C	500	30	510	33	590	48	600	40	580	30	560	27	540	23	510	22
12. 1.0% Aerosol OT	480	26	480	29	510	34	550	40	570	38	560	33	550	30	540	29
13. 1.0% Ammonyx T	540	35	630	37	550	22	480	15	—	—	—	—	—	—	—	—
14. 1.0% Acidol A	530	35	580	33	510	18	440	13	—	—	—	—	—	—	—	—

chain anionic compounds containing the sulfate or sulfonate group. A comparison of data given in Table I for sodium laurate (curve 5) and sodium lauryl sulfate (curve 11) illustrates the differences between the $RCOO^-$ and the more strongly negative RSO_3^- and $ROSO_3^-$ groups. Nacconol (sodium alkyl aryl sulfonate) exhibited the most striking changes of any anion compound thus far examined.

Examples of two nonionic compounds, Tween 80 and Triton X-100, are shown in Table I, curves 7 and 8, and illustrated by Fig. 2, curve 8. No effect on the curve pattern is discernable.

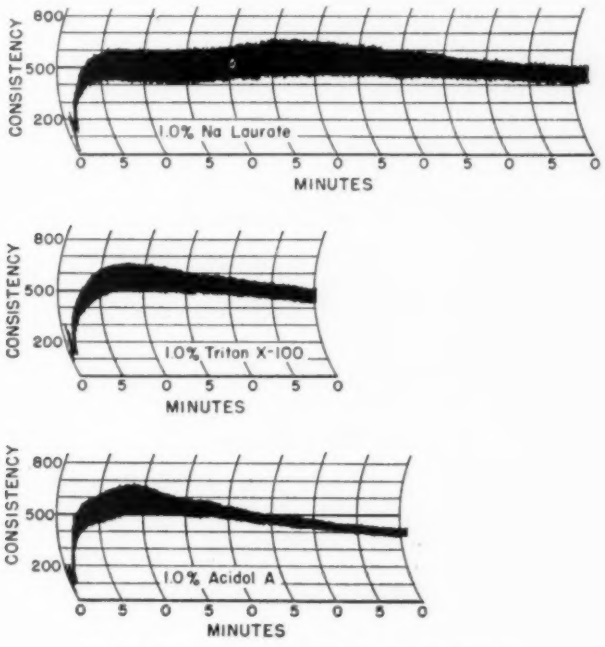

FIG. 2. Top, curve 5; Center, curve 8; Bottom, curve 14.

Several cationic detergents were tested and farinograph data for two, Ammonyx T and Acidol A, are given in Table I. Figure 2, curve 14, illustrates a typical curve for a cationic surface active compound. Cationic detergents show comparatively little effect except for a slight weakening of the curve as shown by a slightly faster breakdown and a narrower amplitude.

Baking Characteristics. Because of the effect of anionic detergents in increasing the mixing time and amplitude of flour-water curves, several baking tests were conducted on both winter and spring wheat

flours to ascertain if these evidences of increased strength were reflected in improved baking characteristics. One such series of tests, using a hard winter wheat flour of 0.43% ash and 11.50% protein with various amounts of Nacconol in a straight dough, is illustrated in Table II. In general, the dough became slightly softer and wetter and there was a definite trend toward decreased volume with increasing amounts of Nacconol regardless of the mixing time. When commercial size sponge doughs were employed with spring wheat flour, no improvement in tolerance or dough-handling characteristics was evident using 0.1% Nacconol. The dough containing the detergent was somewhat tougher,

TABLE II

EFFECT OF NACCONOL ON BAKING CHARACTERISTICS OF A BLEACHED AND MALTED HARD WINTER WHEAT PATENT [1]

Treatment	Gassing Power, mm. 6th Hour	Dough Quality	Loaf Volume, % of Standard	Grain and Texture	Crust Color
Control, no Nacconol 0.65% malt flour	490	Elastic, sl. soft	100	100	Good
0.05% Nacconol 0.65% malt flour	497	Elastic, sl. soft	99	100	Good
0.10% Nacconol 0.65% malt flour	494	Elastic, sl. soft	95	100	Slightly pale
0.15 Nacconol 0.65% malt flour	493	Elastic, soft	94	100	Slightly pale
0.10% Nacconol 1.30% malt flour	530	Elastic, sl. soft	96	100	Good
0.15% Nacconol 2.00% malt flour	547	Elastic, very soft	98	99	Dark

[1] An average commercial formula (2.0% yeast, 2.0% salt, 4.0% sugar, 3.0% shortening, and 0.25% yeast food) was employed in a straight dough procedure.

as might be expected, but was wetter in the overhead proof. Loaf volume was the same and grain and texture somewhat improved as compared with the control.

Flour Paste Viscosity. Since the crust color of the loaves made from the flour containing 0.1%, or more, Nacconol was paler, it was evident that this compound might inhibit the action of the amylases although this was not apparent from gassing power determinations. Marnett and Selman (3), working with another type (nonionic) of surface active agent, showed that polyoxyethylene monostearate increased the maximum paste viscosity as measured on the amylograph and that a significant increase in maximum viscosity was produced in

the absence of alpha-amylase. Figure 3 illustrates the amylograph and gassing power results given by a malted, spring wheat, straight grade flour with varying levels of Nacconol. Further investigation showed that, at levels much above 0.1% of the weight of the flour, all types of surface active agents—anionic, cationic, and nonionic—increased the paste viscosity as measured in the usual manner on the amylograph. For example, a cationic detergent, cetyl trimethyl ammonium bromide, when employed at a level of 1.0%, caused greater increase in paste viscosity than 1.0% Nacconol. An indication that inhibition of alpha-amylase may be involved, in the case of at least some of the surface active agents, is suggested because more malt flour is required to obtain the same crust color when such surface agents are used in the dough.

Reduction of Iodate. When the method described by Howe (1) for the measurement of potassium bromate in flour was employed with flour containing 50 p.p.m. potassium iodate, it was found that, unlike bromate, most of the iodate was reduced. Iodate is reduced at a higher pH than bromate. When, however, the same flour containing 50 p.p.m. potassium iodate and Nacconol was measured, the flour containing the Nacconol showed less reduction of the iodate, indicating that the Nacconol may bind the reducing groups of the flour. At a 5.0% level, Nacconol showed less reduction than at the 1.0% level. The results were as follows:

	Treatment		
Sample	*Potassium Iodate p.p.m.*	*Nacconol %*	*Iodate Reduced %*
Spring wheat straight	50	None	61
Spring wheat straight	50	1.0	48
Spring wheat straight	50	5.0	28

Discussion

Any means whereby some insight can be gained concerning the nature of the forces operating in cross-linking the peptide chains of gluten and in conjugating other substances such as lipids and carbohydrates is of fundamental value in understanding the differences in the quality of flours. Surface active agents are useful tools for this purpose.

Of all the surface active agents used in the experiments described, as well as numerous others, only the anion-active compounds showed the phenomenal increase in mixing time and amplitude and the decreased rate of breakdown on the farinograph curve. Since all curves were made at the normal flour-water pH of 5.9, it would not be antici-

pated that cationic surface active compounds would show much effect because they would be expected to form complexes with acid groups of the proteins, such as carboxy, phenolic, and sulfhydryl, at pH's far on the alkaline side. At pH 5.9, both nonionic and cationic compounds showed little, or no, effect on the farinograph curves except in the direction of weakening the curves at the 1.0% level.

The main interaction of anion-active compounds appears to be with the protein component of flour since gluten itself exhibits changes in physical properties that would be expected from the curve. Removal of fat from flour by ethyl ether extraction only served to intensify the effect of anionic compounds.

It would be expected that anions would combine most readily with proteins that have basic properties and, in many instances, this has

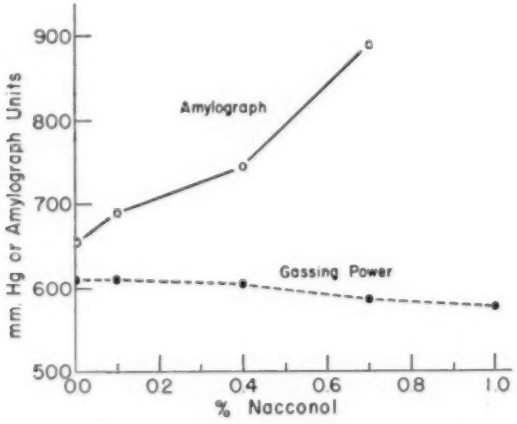

FIG. 4. Effect of Nacconol on gassing power and amylograph viscosity.

proved to be the case. The proteins of flour, however, are notably low in lysine and other basic amino acids. They are unusually high in amide groups as indicated by the ammonia formed during hydrolysis. Such nonionic polarizable groups as the amide could conceivably combine with ionic groups of other molecules by means of electrostatic forces although the amide groups would not carry a strongly positive charge as would a free amino group.

Measurements of the extent of anion binding are difficult because gluten is an insoluble, heterogeneous protein containing both lipids and carbohydrates and the usual criteria, such as the determinations of molecular weight, of viscosity, and electrophoresis, cannot be used.

The explanation of gluten quality and of physical dough characteristics, such as elasticity, toughness, shortness, and extensibility, is

to be found in the mode of cross-linking of the linear polypeptide chains of the flour proteins. The study of the binding of anionic compounds with the proteins of flour promises to shed some light on this most interesting problem of cereal chemistry.

Literature Cited

1. HOWE, MARJORIE. A rapid method for the determination of potassium bromate in flour. Cereal Chem. **28:** 132–135 (1951).
2. LUNDGREN, H. P. Synthetic fibers made from proteins. *In* Advances in Protein Chemistry **5:** 305–351. Academic Press, Inc., New York, N. Y. (1949).
3. MARNETT, L. F., and SELMAN, R. W. Studies of the effects of polyoxyethylene monostearate on flour amylase activity. Cereal Chem. **27:** 349–358 (1950).
4. PUTNAM, F. W. The interactions of proteins and synthetic detergents. *In* Advances in Protein Chemistry **4:** 79–122. Academic Press, Inc., New York, N. Y. (1948).
5. STEINHARDT, JACINTO. The chemistry of the amino acids and proteins. Ann. Review of Biochemistry. **14:** 145–174 (1945). Annual Reviews, Inc., Stanford University, P. O., California.
6. SULLIVAN, ELIZABETH, J. Accelerated moisture conditioning and milling of grain. U. S. Patent 2,413,472. December 31, 1946.
7. SWANSON, C. O., and ANDREWS, A. C. Factors which influence the physical properties of dough. IV. The effects of surface active agents on the characteristics of the curves made by the recording dough mixer. Cereal Chem. **19:** 102–120 (1942).
8. SWANSON, C. O., and ANDREWS, A. C. Factors which influence the physical properties of dough. V. Gluten protein as the main factor affecting the pattern of mixograms. Cereal Chem. **20:** 61–78 (1943).

EFFECT OF REDUCING AGENTS ON GLUTEN PROTEINS[1, 2]

JAMES W. PENCE and HAROLD S. OLCOTT

ABSTRACT

The addition of monothioglycol to alcoholic or dilute acetic acid, heat-treated dispersions of gluten or gliadin caused the following changes in viscosity: With low concentrations the viscosity dropped and remained low. With higher concentrations, the viscosity dropped, then rose gradually and continuously until the solutions formed opaque gels. These gels could be liquefied with urea. During reduction in the presence of urea, the viscosity fell as usual and then rose to a limited extent; gels did not form. Iodoacetamide prevented the rise in viscosity. Variations in the pH, salt content, and protein concentration of the solutions had a definite but not critical effect on the rates and extents of the viscosity changes.

Molecular weight (by osmotic pressure) and cystine determinations suggest that the protein molecules are fragmented by the reducing agent to produce the initial drop in viscosity. The denatured particles then associate by hydrogen bonding, probably through their sulfhydryl groups.

The dramatic effects of small amounts of oxidizing and reducing agents on bread doughs has long been known, but the mechanism of the action has not yet been established satisfactorily. Oxidizing agents cause a tightening or toughening of the dough; reducing agents cause softening. One group of investigators has attributed these effects to inhibition and activation, respectively, of a proteolytic enzyme in flour that requires free sulfhydryl groups for maximum activity (1, 8). Others have considered the experimental data to show that the reagents may act directly upon the flour proteins (2, 6, 17). Evidence that reducing agents can cause marked changes in gluten in the absence of enzymes was presented by Olcott, Sapirstein, and Blish (14), who heated gluten dispersions in dilute acetic acid to destroy enzyme activity, and found that the addition of reducing agents to such heated dispersions caused an immediate and marked decrease in viscosity. It was later observed that, depending upon the concentration of reducing agent used, the initial viscosity decrease was sometimes followed by an increase in viscosity. Under some conditions the solutions eventually gelled. The present paper is concerned with descriptions of these experiments.

[1] Manuscript received December 11, 1951. Contribution from the Western Regional Research Laboratory, one of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

[2] Presented in part before the Division of Biological Chemistry at the 115th meeting of the American Chemical Society, San Francisco, California, March 27 to April 1, 1949.

Materials and Methods

Gluten was obtained by hand-washing flour doughs essentially by the method of Dill and Alsberg (5). During the course of this work, samples were obtained from a number of different types of flour. No marked differences were found in their behavior toward reducing agents. Most of the data presented were obtained with gluten from high-protein, unbleached flours. The protein was dispersed in the various solvents by means of a Waring Blendor. Starch was removed by centrifugation. Enzymes were destroyed in dilute acetic acid dispersions by the heat treatment previously described (14).

Gliadin preparations were obtained by fractional precipitation either with salt or alkali from gluten dispersions in dilute acetic acid. The gliadin fractions were those remaining in solution after the "glutenin" was precipitated. The amide nitrogen values corresponded to those usually reported for gliadin (3).

Monothioglycol was used as the reducing agent for most of the experiments. Viscosities were determined in modified Ostwald viscometers standardized against glycerol solutions and immersed in a constant-temperature water bath at 25°C. Five milliliters of protein solution were placed in the reservoir of the viscometer, and after initial readings were obtained, the reducing agent was added. Results are expressed in terms of relative viscosity; i.e., the ratio of the outflow time of the protein solution to that of the solvent. Cystine was determined by the method of Vassel as modified by Mecham (11). The osmotic pressure measurements were obtained essentially as described by Bull and Currie (4).

Results

Effect of Concentration of Reducing Agent. The effect of varying the concentration of monothioglycol on the behavior of acetic acid dispersions of gliadin is shown in Fig. 1. With the least amount used there was an immediate drop in viscosity which thereafter did not change appreciably. With successively larger amounts the viscosity first dropped and then rose, both the rapidity of the drop and the subsequent rise being greater with the increased concentration. The increase in viscosity was apparently linear with time. All solutions in which the viscosity continued to rise eventually gelled.

Effect of pH. The rates of viscosity changes during reduction of gliadin dispersed in 50% alcohol were markedly affected by pH (Table I). At low pH values the changes in viscosity were much slower. Approximately the same minimum viscosity was obtained at all pH levels.

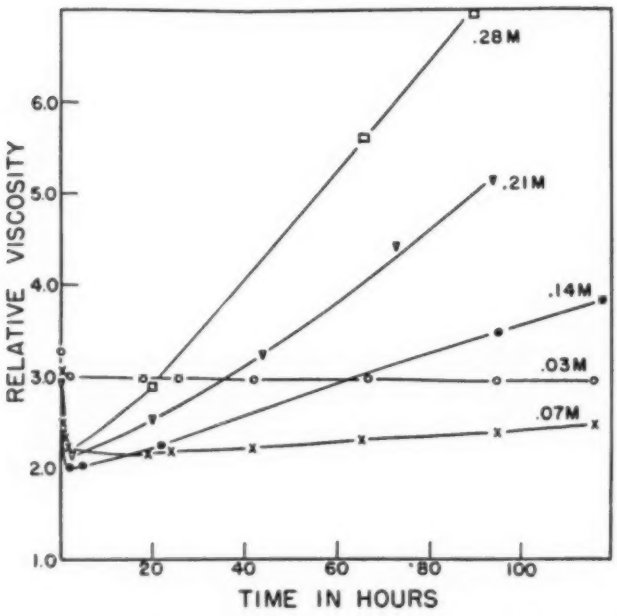

FIG. 1. Effect of varying concentrations of monothioglycol on the viscosity of gliadin dispersions in 0.1 *M* acetic acid.

TABLE I

THE EFFECT OF pH UPON THE RATES OF VISCOSITY CHANGE IN ALCOHOLIC GLIADIN DISPERSIONS REDUCED WITH MONOTHIOGLYCOL[1]

pH	Initial Relative Viscosity	Minimum Relative Viscosity	Time to Minimum Viscosity	Secondary Viscosity Behavior
			hrs.	
2.2	1.81	1.75	30	No viscosity increase in 8 days.
3.4	1.82	1.78	<24	Slight viscosity increase in 8 days
4.4	1.82	1.77	3	Viscosity doubled in 8 days
5.6	1.80	1.77	1	Gel formed in 24 hours
6.8	1.86	1.79	0.5	Flocculation at 2 hours
8.5	1.91	—	None observed	Gel formed in 1 hour

[1] 4.5% solution of gliadin in 50% alcohol solution; pH adjusted with hydrochloric acid and sodium hydroxide. Monothioglycol concentration, 0.14 *M*.

Effect of Salts, Urea, etc. Various neutral salts were found to suppress the viscosity changes to a limited and variable extent. Such salts could be added directly to alcoholic gliadin dispersions, but with acetic acid dispersions of gliadin or gluten, urea was also added to prevent precipitation of the proteins by the salts. In the presence of urea, reduction led to the usual viscosity decrease, but the following rise in viscosity was severely limited so that high values were never obtained.

Gel formation was readily obtained with gliadin dispersed in 50% alcohol, if either monothioglycol or bisulfite (to final concentration, approximately 0.3 *M*) was used as the reducing agent. When cysteine was used, gradual precipitation of the protein occurred without a visible decrease in viscosity. Urea (3 *M*), guanidine hydrochloride (1 *M*), or alkali (0.1 *M*) readily liquefied the gels formed in alcohol, but Duponol, Nacconol, thiourea, acids, or mild heating did not.

Properties of Reduced Gliadin and Gluten. Gliadin gels which were liquefied by urea again formed gels after the urea had been removed by dialysis, although it was sometimes necessary to use additional reducing agent at this point, perhaps to counteract re-oxidation. Liquefied gels treated with iodoacetamide, which reacts irreversibly with sulfhydryl groups (7, 13), did not form gels as readily upon subsequent removal of urea. Iodoacetamide added to a reduced alcoholic gliadin dispersion (pH 6.0–6.2) at the point of minimum viscosity entirely quenched the subsequent viscosity rise. Potassium bromate was much less effective and a portion, at least, of its action probably could be attributed to its effect as a salt. These observations suggest that the sulfhydryl group participates in the viscosity-increasing mechanism.

A small but real increase in dialyzable nitrogen (2–5%) was found with reduced gluten and gliadin. Osmotic-pressure determinations showed that the apparent molecular weight of gliadin dispersed in 10 *M* urea was not changed significantly by reduction, but reduction of gluten dispersed in 6 *M* guanidine hydrochloride produced a decrease in apparent molecular weight from about 42,000 to about 25,000. Ultracentrifuge studies on reduced and un-reduced gluten dispersed in 6 *M* guanidine hydrochloride showed that the sedimentation constant was lowered from $S_{20} = 1.03$ to 0.72 upon reduction.

Cystine Analyses. By alkylation (with iodoacetamide) of free sulfhydryl groups following reduction (13), a decrease in total cystine content could be shown to accompany the decrease in viscosity of gliadin and gluten dispersions. The cystine content continued to fall even though the viscosity had reached a minimum and had begun to rise. In contrast, the viscosity decrease produced by the naturally occurring proteinases in a raw gluten dispersion was not accompanied by a decrease in cystine content, thus indicating a fundamental difference in the two mechanisms (Table II).

The total cystine (plus cysteine) contents of gluten or other proteins, however, were unreliable criteria of a susceptibility to a decrease in viscosity during reduction. The cystine content after reduction was lowered as much in gliadin as it was in gluten, but the viscosity decreases were not as marked. Table III shows the viscosity changes resulting from the reduction of several proteins. Casein showed prac-

TABLE II

COMPARISON OF CHANGES IN RELATIVE VISCOSITY AND CYSTINE CONTENT IN UNHEATED AND HEATED, REDUCED GLUTEN DISPERSIONS

Gluten Dispersion	Time	Relative Viscosity	Cystine Content
	hrs.		%
Unheated[1]	0	5.1	1.9
	80	2.7	1.8
	860	—	1.9
Heated and reduced[2]	0	5.7	1.9
	2	2.5	1.8
	19	2.3	1.2
	42	2.3	1.1
	76	2.5	0.9
	139	2.6	0.9

[1] Gluten dispersion in 0.1 *M* acetic acid, pH 3.5. Drop in viscosity is due to presence of flour enzyme (14).
[2] Same dispersion heated at 95°C. for 3 minutes to destroy enzymes, then plus monothioglycol to 0.28 *M*.

tically no changes. Zein showed a very small decrease in viscosity, followed by only a slight increase. Bovine serum albumin (6% cystine) showed no detectable decrease in viscosity, but did exhibit a very rapid increase in viscosity. In this case a viscosity-increasing mechanism might have obscured the viscosity-decreasing effects. However, reduction of this protein in urea caused no decrease in viscosity. Insulin has been reported to show no decrease in viscosity after reduction, but its solutions soon set to heavy, thixotropic gels (16). Lima bean trypsin inhibitor, although it contains about 15% cystine, showed practically no change in viscosity when reduced, but a flocculent precipitate

TABLE III

COMPARISON OF EFFECTS OF MONOTHIOGLYCOL (0.28*M* FINAL CONC.) ON VARIOUS PROTEINS

Protein[1]	Approx. Conc.	Approx. pH	Cystine Content	Viscosity Behavior
	%		%	
Gliadin	5	3.5	2.4	Small decrease, large inc.
Gluten	4	3.5	1.8	Large decrease, mod. inc.
Glutenin	3	3.5	1.3	Large decrease, mod. inc.
Casein	1	6.5	0.4	No changes
Zein	5	5.5	0.8	Small changes
Bovine serum albumin	5	4.0	6.5	No decrease, large rapid inc.
Insulin[2]	5	2.0	12.4	No decrease, large rapid inc.
Lima bean trypsin inhibitor	4	6.0	15.0	No decrease, small increase to flocculation

[1] Dispersion media were 0.1 *N* acetic acid for gliadin, gluten, glutenin, and bovine serum albumin; 0.008 *M* phosphate buffer for casein; 70 per cent ethanol for zein; 0.008 *M* thioglycolic acid for insulin; and distilled water for lima bean trypsin inhibitor.
[2] Data of Stern and White (16).

soon appeared. Reduction of this protein in urea solution showed only a slow, limited increase in viscosity.

Peracetic Acid Oxidation. Organic peracids have been shown to oxidize readily only tryptophan, methionine, and cystine (18) and have been used to split the disulfide bonds of insulin without damaging the rest of the molecule (15). The effect of peracetic acid oxidation upon the viscosity behavior of gluten and gliadin dispersions was compared with the results obtained with reducing agents. The acid was formed *in situ* by adding 1 ml. of 30% hydrogen peroxide to 4 ml. of a 0.1 *N* acetic acid dispersion of the proteins. The peracetic acid caused only a very slight decrease in the viscosity of gliadin dispersions followed by a very slight increase before precipitation of the protein occurred. The cystine content of the protein, however, was lowered from 2.4 to 0.5% by the oxidation, showing that the disulfide bonds were extensively affected.

Gluten dispersions, which exhibit much greater viscosity decreases with reducing agents than those of gliadin, likewise showed a very large drop in viscosity during treatment with peracetic acid. Gradual precipitation of the protein rather than gel formation occurred also with this protein.

Solutions of bovine serum albumin failed to show a decrease in viscosity after oxidation with peracetic acid, but a clear, firm gel formed after a long period of slowly increasing viscosity.

Discussion

The viscosity data obtained in the present experiments indicate that reducing agents cause either an initial contraction or fragmentation of the gluten protein molecules. Although the high concentrations and complexity of the proteins employed make physico-chemical treatment of these data of doubtful value as a means to resolve these possibilities, the osmotic-pressure molecular-weight determinations and the sedimentation data both suggest that fragmentation is the probable effect of the reducing action. The viscosity curves obtained appear to be the result of the opposing tendencies of fragmentation, which would lower the viscosity, and of unfolding and aggregation of the reduced particles, which would increase it.

The ability of reducing agents to render proteins insoluble has previously been noted by others for insulin, crotoxin, lysozyme, and lactogenic hormone, and the insolubility has been attributed to the formation of new hydrogen bonds presumably involving sulfhydryl groups (7, 13). More recently Huggins *et al.* (9, 10) have shown that mercaptans can coagulate bovine plasma albumin, γ globulin, and egg albumin, and that urea prevents the coagulation. The experiments

with gluten furnish additional evidence that sulfhydryl groups are involved in these phenomena, since blocking them with specific reagents prevents gelation or precipitation.

Acknowledgments

We are indebted to Wilfred Ward of this Laboratory for the ultracentrifuge examinations and to Ali Mohammad for the osmotic-pressure determinations.

Literature Cited

1. Balls, A. K., and Hale, W. S. Proteolytic enzymes of flour. Cereal Chem. **13:** 54–60 (1936).
2. Balls, A. K., and Hale, W. S. Further studies on the activity of proteinase in flour. Cereal Chem. **13:** 656–664 (1936).
3. Blish, M. J. Wheat gluten. Advances in Protein Chem. *II:* 337–359 (1945).
4. Bull, H. B., and Currie, B. T. Osmotic pressure of beta-lactoglobulin solutions. J. Am. Chem. Soc. **68:** 742–745 (1946).
5. Dill, D. B., and Alsberg, C. L. Some critical considerations of the gluten washing problem. Cereal Chem. **1:** 222–246 (1924).
6. Ford, W. P., and Maiden, A. M. The effects in dough of glutathione and papain. Trans. Soc. Chem. Ind. **57:** 278–281 (1938).
7. Fraenkel-Conrat, H., Mohammad, A., Ducay, E. D., and Mecham, D. K. The molecular weight of lysozyme after reduction and alkylation of the disulfide bonds. J. Am. Chem. Soc. **73:** 625–627 (1951).
8. Jørgensen, H. On the existence of powerful but latent proteolytic enzymes in wheat flour. Cereal Chem. **13:** 346–355 (1936).
9. Huggins, C., Tapley, D. F., and Jensens, E. V. Mercaptan-induced coagulation of proteins. Proc. Nat'l. Acad. Sci. **36:** 695–699 (1950).
10. Huggins, C., Tapley, D. F., and Jensens, E. V. Sulfhydryl-disulphide relationships in the induction of gels in proteins by urea. Nature **167:** 592–593 (1951).
11. Mecham, D. K. The determination of cysteine and cystine by Vassel's method. J. Biol. Chem. **151:** 643–645 (1943).
12. Olcott, H. S. Stabilization of solutions of wheat gluten in dilute acetic acid by brief heat treatment. Cereal Chem. **27:** 514–516 (1950).
13. Olcott, H. S., and Fraenkel-Conrat, H. Specific group reagents for proteins. Chem. Rev. **41:** 151–197 (1947).
14. Olcott, H. S., Sapirstein, L. A., and Blish, M. J. Stability of wheat gluten dispersions toward reducing agents in the presence and absence of a gluten proteinase. Cereal Chem. **20:** 87–97 (1943).
15. Sanger, F. Oxidation of insulin by performic acid. Nature **160:** 295–296 (1947).
16. Stern, K. G., and White, A. Studies on the constitution of insulin. I. Properties of reduced insulin preparations. J. Biol. Chem. **117:** 95–110 (1937).
17. Sullivan, B., Howe, M., Schmalz, F. D., and Astleford, G. R. The action of oxidizing and reducing agents on flour. Cereal Chem. **17:** 507–528 (1940).
18. Toennies, G., and Homiller, R. P. The oxidation of amino acids by hydrogen peroxide in formic acid. J. Am. Chem. Soc. **64:** 3054–3056 (1942).

GRAIN STORAGE STUDIES XI. LIPOLYTIC ACTIVITY OF FUNGI ISOLATED FROM STORED CORN [1]

J. J. GOODMAN [2] and CLYDE M. CHRISTENSEN [3]

ABSTRACT

Single cultures of four species of fungi, *Penicillium solitum*, *Aspergillus flavus*, *A. candidus*, and *A. amstelodami*, isolated from moldy stored corn, were grown on corn meal from which the oil had been extracted, and on similar meal to which the oil originally extracted had again been added. All caused an initial increase in fatty acids in the meal containing oil, but after having reached a peak the fat acidity decreased. The amount of fatty acids produced varied with the species. None of the fungi caused any increase in fatty acids in the oil-free meal. In these tests the fatty acids were produced as a result of fungus lipases acting upon the corn oil. No differences were noted in the amount of water soluble acids produced in oil-free meal and in meal containing oil. Dried mycelium of the four species, as well as that of one isolate of *Aspergillus terreus* from moldy corn, contained active lipase. Three of the isolates contained much more lipase when grown on a medium containing corn oil than when grown on a medium containing sucrose as the carbohydrate source. The production of various acids from sucrose by these fungi, as determined on indicator media, bore no relationship to their lipolytic activity. All five of the isolates were able to use two or more of the constituent fatty acids of corn oil as sources of carbon.

It is now fairly well established that fungi are mainly responsible for the deterioration of grains stored with excess moisture (5, 14, 19, 24). A widely used chemical index of such deterioration is the increase of fat acidity in stored grain. It has been suggested that fat acidity arises from the action of seed lipases (1, 8, 16, 17) but it may also arise from the action of the fungi on seed constituents. Autoclaved grain inoculated with various storage fungi exhibits increases in fat acidity similar to those found in stored viable grain (5, 20, 21). There is often a fairly close correlation between increases in fat acidity and increases in mold count of stored grain (4, 6, 21, 25). Fungi would thus seem to play a more dominant role in acidity increases than do seed lipases.

[1] Manuscript received November 27, 1951. Paper No. 2773, scientific journal series article, Minnesota Agricultural Experiment Station. This material constituted a portion of a thesis presented by the Senior Author to the Graduate School, University of Minnesota, in partial fulfillment of the requirement for a Ph.D. degree.

[2] Research Assistant in Plant Pathology. Present address: Lederle Laboratories Division, American Cyanamid Co., Pearl River, New York.

[3] Professor of Plant Pathology, Division of Plant Pathology and Botany, Agricultural Experiment Station, University of Minnesota, St. Paul 1, Minnesota.

The mechanisms whereby fungi increase fat acidity are not well established. The genera most frequently encountered, Penicillium and Aspergillus, are known to contain species that produce lipase (9, 10, 11, 17, 23, 26) as well as a variety of organic acids (12, 29). Nagel and Semeniuk (21) found various species to differ in their ability to increase fat acidity of autoclaved corn. They found a general agreement between the pH and fat acidity developed on corn and the pH and titratable acidity developed in sucrose media. This led them to suggest that part of the measured fat acidity might be due to metabolic acids produced by the fungi from corn carbohydrates.

This report deals with the origin of the fat acidity produced by fungi in grain, the growth of the fungi on fats and fatty acids and their lipase activity in synthetic media. Five species of fungi isolated during routine assays of molded corn were used. These were *Penicillium solitum* Westling, *Aspergillus flavus* Link, *A. terreus* Thom, *A. candidus* Link and an isolate of the *A. glaucus* group identified as *A. amstelodami* (Mangin) Thom and Church.

Origin of Fat Acidity in Molded Corn

Whole sound corn was ground in a hand mill and then extracted with several changes of petroleum ether over a period of three to four days. The extracts were bulked and the solvent distilled off. One-half of the residual corn oil was added to one-half of the extracted meal while the rest of the meal was left extracted. The two types of meal were then adjusted to the desired moisture content, dispensed in 50 g. amounts into 4 oz. medicine bottles and sterilized. The bottles were inoculated by adding 0.5 ml. of a spore suspension of the fungus after which they were shaken vigorously to distribute the inoculum. Incubation was carried out at room temperature (20°C. to 23°C.). Fat acidity was determined by the short method outlined in Cereal Laboratory Methods (2) and is expressed as milligrams of potassium hydroxide required to neutralize the benzene extract from 10 g. of corn on a wet weight basis. Water-soluble acids were assayed by removing a 20 g. aliquot of meal, adding 150 ml. of water, shaking in a mechanical shaker for one hour and then titrating 75 ml. of the liquid with a standard solution of potassium hydroxide.

In the first test the meals were adjusted to 20% moisture (wet weight), and inoculated with *P. solitum*, *A. flavus*, *A. candidus* and *A. amstelodami*. At ten days after inoculation, a 20 g. aliquot was removed from each of three bottles and the fat acidity determined. The bottles were replaced and a second aliquot was removed on the twenty-first day for the determination of fat acidity. Results are presented in Table I.

TABLE I

MILLIGRAMS OF POTASSIUM HYDROXIDE REQUIRED TO NEUTRALIZE THE BENZENE EXTRACT OF 10 G. OF FATTED AND DEFATTED GROUND CORN STORED AT 20% MOISTURE

Fungus	10 Days		21 Days	
	Minus Fat	Plus Fat	Minus Fat	Plus Fat
Uninoculated	1.2[1]	3.0	contaminated [2]	
P. solitum	2.7	16.2	4.1	4.8
A. flavus	3.3	16.8	4.6	15.2
A. candidus	2.9	8.8	3.6	4.6
A. amstelodami	3.2	9.8	5.2	7.3

[1] Each figure is the mean of 3 replicates
[2] After the first aliquot was removed, the non-inoculated meal became contaminated due to opening the bottles. The inoculated meals already bearing a vigorous mold growth did not become visibly contaminated.

In the second test the meals were brought to 30% moisture (wet weight) and inoculated with *P. solitum*, *A. flavus* and *A. amstelodami*. Three different replicate bottles were removed every five days and the meal assayed for both fat acidity and water-soluble acids. The results are given in Table II.

TABLE II

MILLIGRAMS OF POTASSIUM HYDROXIDE REQUIRED TO NEUTRALIZE THE BENZENE EXTRACT OF 10 G. OF FATTED AND DEFATTED GROUND CORN STORED AT 30% MOISTURE

Fungus and Type of Corn	Days After Inoculation					
	0	5	10	15	20	25
Uninoculated						
Minus Fat	2.1	1.8	3.0	1.8	—	2.7
Plus Fat	3.8	3.8	4.6	4.0	—	5.1
P. solitum						
Minus Fat	2.1	3.1	3.6	1.7	1.2	5.2
Plus Fat	3.8	27.9	47.8	33.8	23.6	17.0
A. flavus						
Minus Fat	2.1	2.1	2.1	2.9	2.8	3.6
Plus Fat	3.8	10.2	6.2	28.3	16.3	16.7
A. amstelodami						
Minus Fat	2.1	2.0	1.4	1.5	0.7	0.4
Plus Fat	3.8	4.0	12.9	49.2	31.8	19.0

In both experiments, the meal in all inoculated bottles, with or without oil, supported an equally heavy growth of mold. All of the meals were thoroughly matted with a pure culture of fungus mycelium at the end of the test. Without fat, despite heavy mold growth, the fat acidity remained at a constant low level, whereas, in the meals

containing fat, the acidity rose to a peak then fell with time. Preliminary experiments made on fatted and defatted inoculated wheat germ have indicated similar trends in these materials. Nagel and Semeniuk (21) and Bottomley, Christensen and Geddes (4) also found similar results. The latter investigators suggested that the decreases in acidity were possibly due to the metabolism of the acids by the fungi.

The data for water-soluble acids were more erratic than those for fat acidity due to the difficulty of reading end points. For all of the fungi these acidity levels rose only slightly then decreased to the initial levels. There were no appreciable differences in the amount of water-soluble acids in fatted and defatted meals.

Though fat acidities were determined on ground and extracted meals, the results should be applicable to whole grain stored under unfavorable conditions but in whole grain the changes in acidity would not be so rapid. In these tests, the rise in fat acidity which accompanied fungus infestation was derived from the action of fungus lipases upon fats present in the meal itself. Since water-soluble acids in fatted and defatted meals were similar, the present method of measuring fat acidity (2) actually does measure the fat acidity and not other acids which might be elaborated by the fungi from corn carbohydrates as has been suggested (21).

Except under extremely unfavorable conditions such as might exist in the upper layers of storage bins (25) one would not find as matted a growth of fungi as was found in the experiments here reported. Under commercial conditions fat acidity values would seldom approach those attained in these tests. In any case fat acidity under those conditions would be an unnecessary criterion of deterioration since the grain would be visibly moldy and would be graded as such. Under experimental conditions, however, fat acidity is an index which must be evaluated with regard to length of storage. There is an initial sharp rise in fat acidity followed by a decrease. A more "deteriorated" sample may thus actually have a lower fat acidity.

Growth of Fungi on Fatty Acids

It has been suggested that the fall in fat acidity with time in molded grain might be due to the utilization of the fatty acids by the fungi (4). Tests were made to determine if this could occur.

The fungi were tested in Czapek-Dox medium (28) for their ability to utilize corn oil, synthetic monoglycerides and the constituent fatty acids of corn oil as sources of carbon. These various sources were used at levels to give approximately the same amount of carbon as in 3% sucrose. The water-insoluble ingredients were incorporated into the media by passing them through a hand-operated homogenizer several

times. As a stabilizing agent 0.25% agar was added to all the media. This resulted in a semi-solid medium from which the mycelial mats could easily be removed. The fungi were also tested on non-toxic levels of the sodium soaps of the acids. The amount of carbon in these cases was less than that present in a 3% sucrose medium. Fifty ml. of medium was dispensed into 250 ml. Erlenmeyer flasks; sterilized and inoculated with 0.5 ml. of a spore suspension of the fungus. The fungi were allowed to grow at room temperature (20° to 23°C.). After a period of growth the mycelial mats were removed, washed in several changes of cold water and dried for 24 hours at 95°C. The average dry weight of mycelium from three replicate flasks was used as a measure of the amount of growth.

It will be seen from Tables III, IV, and V that all of the fungi tested are able to utilize to some extent either corn oil, a monoglyceride and fatty acids. The amount of growth on the fats was much less than on sucrose; still an appreciable amount of growth was achieved on corn oil and on stearic and oleic acids. The amount of growth on the soaps cannot be directly compared to the growth on corn oil or on the acids, as they had to be tested at a non-toxic level. Even so, the fungi grew on the soaps as a sole carbon source.

TABLE III

AVERAGE DRY WEIGHT OF MYCELIUM OF FUNGI GROWN ON DIFFERENT CARBON SOURCES

Fungus	Corn Oil 1.5%	Glyceryl Mono-Oleate[1] 1.5%	Sucrose 3%
	mg.	*mg.*	*mg.*
P. solitum	132 (14)[2]	67 (15)	738 (8)
A. flavus	199 (14)	97 (15)	445 (8)
A. terreus	280 (14)	131 (15)	586 (8)
A. candidus	90 (20)	45 (15)	390 (13)
A. amstelodami	0 (20)	trace (15)	258 (13)

[1] S-1097. A product obtained through the courtesy of Glyco Products Co., Inc., Brooklyn, New York.
[2] Figures in brackets refer to age in days when harvested.

TABLE IV

AVERAGE DRY WEIGHT OF MYCELIUM PRODUCED ON FATTY ACIDS[1] IN 14 DAYS

Fungus	Palmitic	Stearic	Oleic	Linoleic
	mg.	*mg.*	*mg.*	*mg.*
P. solitum	36	135	157	0
A. flavus	32	185	432	0
A. terreus	34	207	448	0
A. candidus	28	115	97	0
A. amstelodami	27	0	135	0

[1] Acids were homogenized with gum arabic which by itself did not support any growth. Acids were used in amounts to give the carbon equivalent of 3% sucrose.

TABLE V

AVERAGE DRY WEIGHT OF MYCELIUM PRODUCED ON 0.01 *M* SODIUM SOAPS IN 15 DAYS

Fungus	Sodium Palmitate	Sodium Stearate	Sodium Oleate	Sodium Linoleate
	mg.	*mg.*	*mg.*	*mg.*
P. solitum	17	21	35	30
A. flavus	23	25	41	40
A. terreus	24	24	48	37
A. candidus	19	33	37	0
A. amstelodami	0	0	13	0

Of the five fungi tested, *A. amstelodami* in our experience tended to grow rather poorly on fats. This is not due to any lack of lipase activity (see preceding and following section) but rather to other inadequacies in the media. The supplementation of corn oil media with small amounts of corn steep solids usually permitted good growth. This organism is known to prefer media of high osmotic pressure (28). In our experience the supplementation of liquid media with 6 to 8% sodium chloride also was found to improve its growth on liquid media.

The fall in fat acidity found in the experiments presented in Tables I and II could thus very easily be due to the utilization of the fatty acids produced by the fungi for their own further growth.

Fungus Lipase Activity on Synthetic Media

The first section presented data indicating marked lipase activity in ground corn meal. It was interesting therefore to test for lipase activity both in the presence and absence of growth on synthetic media.

Qualitative tests of the lipolytic activity of the growing fungi were made on two indicator media. The medium of Collins and Hammer (7) containing Nile Blue Sulfate and Berry's medium (3) containing copper sulfate were used to detect lipolysis of corn oil emulsified into the media. Acid production on agar media containing sucrose was also tested by the method of Foster and Davis (13).

Approximately 50 isolates of Penicillium and Aspergillus were tested and though the methods were only qualitative, no consistent relationship was found between the lipolytic activity and the ability to produce acids from sucrose. Individual isolates active in one category were not necessarily active in the other. Results from five isolates used in these experiments are presented in Table VI. None of these were vigorous acid producers on sucrose but most of them were lipolytic to some degree.

TABLE VI

LIPASE ACTIVITY AND PRODUCTION OF ACIDS ON AGAR MEDIA [1]

Fungus	Lipase Activity		
	Nile Blue Sulfate	Copper Sulfate	Acid Production From Sucrose
P. solitum	3	4	1
A. flavus	3	2	1
A. terreus	1	1	1
A. candidus	3	2	1
A. amsteldami	1	0	trace

[1] Lipase and acid producing ability were judged visually on the basis of size and intensity of the colored zones produced around the colonies. Ratings were made as follows: 0 = none, 1 = poor, 2 = fair, 3 = good, 4 = excellent.

Quantitative tests of the lipase activity in the absence of growth were also made. These were carried out by the method of Peters and Nelson (22), in which the acids liberated from buffered corn oil emulsion by the various mycelial preparations were titrated with alcoholic sodium hydroxide solution. Czapek-Dox medium, containing the appropriate carbon source, was used to grow the fungi. After a suitable growth period, the mycelia were harvested and prepared for testing lipase activity by three methods: the acetone method of Tate (27), the glycerol method of Fodor and Chari (11), or they were merely air-dried and ground into powder.

P. solitum and *A. flavus* mycelia were previously reported to contain lipase, the activity of which was greatly increased by prior growth of the mold on a medium containing corn oil (15). The results for three other fungi tested in a similar manner are given in Table VII.

TABLE VII

RELATIVE LIPASE ACTIVITY [1] OF THE MYCELIUM OF THREE FUNGI GROWN ON SUCROSE OR CORN OIL

Fungus	Method of Preparation	Grown on:	
		Corn Oil	Sucrose
A. terreus	Glycerol	3.5	1.7
	Acetone	7.4	3.2
	Air-Dried	5.7	6.5
A. candidus	Glycerol	1.0	0.8
	Acetone	1.7	2.3
	Air-dried	Poor growth	7.1
A. amstelodami	Glycerol	No growth	3.1
	Acetone	No growth	4.6
	Air-dried	No growth	8.5

[1] Average ml. N/20 alcoholic sodium hydroxide solution minus control.

Because *Aspergillus candidus* grew poorly and *A. amstelodami* failed to grow with corn oil as the principal carbon source, another test was conducted in which the media were supplemented with 0.2% corn steep solids. Growth of these two isolates was much better in the supplemented than in the non-suppleemnted corn oil media, but not as well as on similarly supplemented sucrose media. Water suspensions of acetone-treated mycelium (27) of these and other fungi grown on supplemented media showed the lipase activity presented in Table VIII.

TABLE VIII

RELATIVE LIPASE ACTIVITY[1] OF THE MYCELIUM OF FIVE FUNGI GROWN ON CORN OIL OR SUCROSE SUPPLEMENTED WITH CORN STEEP SOLIDS

Fungus	Grown on	
	Corn Oil	Sucrose
P. solitum	11.4	0.7
A. flavus	9.4	0.8
A. terreus	1.2	0.2
A. candidus	0.2	0.4
A. amstelodami	8.8	3.4

[1] Average ml. *N*/20 alcoholic sodium hydroxide solution minus control.

As may be seen from Tables VII and VIII, *Penicillium solitum*, *Aspergillus flavus* and *A. amstelodami* were more lipolytically active than were *A. terreus* and *A. candidus*. The lipase activity of the first three fungi was markedly increased by growing them on a medium containing corn oil (see reference 15 also), but the lipase activity of *A. terreus* and *A. candidus* was not appreciably increased by growing on corn oil.

Discussion

All of the fungi investigated produced some lipase when grown on a synthetic medium. Some of the fungi produced much more lipase when grown on a medium containing corn oil as the sole source of carbon than when grown on a medium in which sucrose was the carbon source. This presumably could be a result of: (1) Adaptive production of lipase in the presence of a substrate which the lipase could hydrolyze; (2) Selection, from an original mixed population, grown on a given substrate, of those strains or biotypes able to metabolize this substrate. If this hypothesis is considered to be reasonable, it must be admitted that isolates of two of the species tested contained no strains or biotypes able to metabolize corn oil effectively; (3) A combination of the above two. In the light of evidence presented, it would seem reasonable to suppose that lipase production by at least several of the fungi which were tested is to some extent an adaptive process.

Several of the fungi were able to utilize corn oil as a sole source of carbon, and to metabolize appreciable amounts of fatty acids from it. All of the five isolates were able to utilize, as a source of carbon, one or more of the four fatty acids tested. In other words, some fungi are able to grow upon corn oil and convert a portion of it into fatty acids, and some of these fungi may be able to use at least some of these fatty acids as food.

The results here reported apply only to those islates which were tested, and under the given experimental conditions. Presumably other isolates of these fungus species, as well as other species of fungi common on stored seeds, might behave somewhat differently. When, as usually occurs in nature, a large number of different fungi, with widely different physiologic capabilities, grow upon stored grain under varying conditions of moisture, temperature, and ecologic association, it is likely to be difficult to measure accurately the amount of fungus activity that has occurred in the grain by measuring the amount of fatty acids or other specific products of fungus metabolism that are present at a specific time.

Literature Cited

1. Altschul, A. M., Karon, M. L., Kyame, Lillian, and Hall, Catherine M. Effect of inhibitors on the respiration and storage of cottonseed. Plant. Physiol. **21:** 573–587 (1946).
2. American Association of Cereal Chemists. Cereal Lab. Methods. 4th Ed. (1941).
3. Berry, J. A. Detection of microbial lipase by copper soap formation. Jour. Bact. **25:** 433–434 (1933).
4. Bottomley, R. A., Christensen, C. M., and Geddes, W. F. Grain storage studies X. The influence of aeration, time, and moisture content on fat acidity, non-reducing sugars and mold flora of stored yellow corn. Cereal Chem. **29:** 53–64 (1952).
5. Carter, E. P., and Young, G. Y. Role of fungi in the heating of moist wheat. U.S.D.A. Circ. No. 838 (1950).
6. Christensen, C. M., Olafson, J. H., and Geddes, W. F. Grain storage studies VIII. Relation of molds in moist stored cottonseed to increased production of carbon dioxide, fatty acids and heat. Cereal Chem. **26:** 109–128 (1949).
7. Collins, M. A., and Hammer, B. W. The action of certain bacteria on some simple tri-glycerides and natural fats, as shown by nile-blue sulfate. Jour. Bact. **27:** 473–485 (1934).
8. Condon, Marjorie Z., Andrews, F. R., Lambou, Madeline G., and Altschul, A. M. Inhibition of heating and lipolysis in seeds. Science **105:** 525–527 (1947).
9. Currie, J. N. Flavor of Roquefort cheese. Jour. Agr. Res. **2:** 1–14 (1914).
10. Eyre, J. C. Cultural studies on the Aspergilli, with special reference to lipase production of strains isolated from stored copra and cacao. Ann. Appl. Biol. **19:** 351–369 (1932).
11. Fodor, P. J., and Chari, A. The ester-hydrolysing enzyme systems of *Aspergillus niger* and of *Penicillium Roqueforti*. Enzymologia **13:** 258–267 (1949).
12. Foster, J. W. Chemical activities of fungi. Academic Press, New York (1949).
13. Foster, J. W., and Davis, H. Detection and occurrence of acid producing fungi. Bull. Torrey Bot. Club **76:** 174–176 (1949).
14. Gilman, J. C., and Semeniuk, G. Mold microflora in stored grain and its role in the deterioration process. Trans. Amer. Assoc. Cereal Chem. **6:** 108–112 (1948).

15. Goodman, J. J. Adaptive production of amylase and lipase by three species of fungi. Science **112:** 176–179 (1950).
16. Karon, M. L., and Altschul, A. M. Effect of moisture and of treatments with acids and alkali on rate of formation of free fatty acids in stored cottonseed. Plant Physiol. **19:** 310–325 (1944).
17. Kirsh, D. Lipase production by *Penicillium oxalicum* and *Aspergillus flavus*. Bot. Gaz. **97:** 321–333 (1935).
18. Kyame, Lillian, and Altschul, A. M. Comparison of respiration, free fatty acid formation, and changes in the spectrum of the seed oil during the storage of cottonseed. Plant Physiol. **21:** 550–561 (1946).
19. Milner, M. Biological factors in damp grain deterioration. Food Tech. **5:** 25–30 (1951).
20. Milner, M., and Geddes, W. F. Grain Storage Studies III. The relation between moisture content, mold growth, and respiration of soybeans. Cereal Chem. **23:** 225–247 (1946).
21. Nagel, C. M., and Semeniuk, G. Some mold-induced changes in shelled corn. Plant Physiol. **22:** 20–33 (1947).
22. Peters, I. I., and Nelson, F. E. Factors influencing the production of lipase by *Mycotorula lipolytica*. Jour. Bact. **55:** 581–591 (1948).
23. Richards, T., and El-Sadek, G. M. The nature and quantity of fatty acids produced in butterfat by the action of micro-organisms. Jour. Dairy Research **16:** 46–52 (1949).
24. Semeniuk, G., and Gilman, J. C. Relation of molds to the deterioration of corn in storage. A review. Proc. Iowa Acad. Sci. **51:** 265–280 (1944).
25. Semeniuk, G., Nagel, C. M., and Gilman, J. C. Observations on mold development and on deterioration in stored yellow dent shelled corn. Iowa Agr. Exp. Sta. Res. Bull. 349 (1947).
26. Stuart, L. S. The production of lipolytic and depilating enzymes by the *Aspergillus flavus-oryzae* group. (Abstr.) Jour. Bact. **29:** 88–89 (1935).
27. Tate, P. On the enzymes of certain dermatophytes or ringworm fungi. Parasitology **21:** 31–54 (1929).
28. Thom, C., and Raper, K. B. Manual of the Aspergilli. Williams and Wilkins, Baltimore (1945).
29. Wolf, F. A., and Wolf, F. T. The fungi. Vol. II. John Wiley and Sons, New York (1947).

A NOTE ON THE EFFECT OF MOLD GROWTH AND INCREASED MOISTURE CONTENT ON THE FREE AMINO ACIDS IN HARD RED SPRING WHEAT [1]

J. E. DeVay

ABSTRACT

Eighteen free amino-acids and an unknown ninhydrin-reacting substance, which disappears during hydrolysis, were found in sound and moldy wheat. In addition, gamma-amino butyric acid was identified only in moldy wheat having a relatively high moisture content. Visual comparisons of chromatograms of sound and moldy wheat indicated changes in the concentrations of certain free amino-acids.

The influence of environmental conditions and mold growth on the biochemical processes in stored grain has been the subject of a number of studies (1, 5, 6).

The purpose of this preliminary work was to determine the effect of increased moisture and mold growth on the free amino-acid content of sound wheat. Hard red spring wheat (Lee), germinating 90% and containing 9.5% moisture, was used. A subsample was conditioned to 19.5% moisture (dry weight basis) and stored for 6 weeks in a sealed container. Common storage molds such as *Aspergillus* spp. and *Penicillium* spp. were observed growing on the wheat after 5 days. At the end of 6 weeks the moldy wheat germinated 40%.

The free amino-acids in 50 g. subsamples of sound and moldy wheat, ground to pass through a No. 40 mesh sieve, were extracted with 400 ml. of 70% ethanol for 1.5 hours at 60°C. After this, the samples were filtered, re-extracted with 200 ml. of distilled water for 1 hour at 60°C., and then filtered and washed with 100 ml. of 70% ethanol. The extracts of each sample were combined and concentrated under reduced pressure at 60°C. to approximately 200 ml. to precipitate most of the alcohol-soluble protein. Each extract was then filtered and concentrated as before to a thick syrup which was made up to 100 ml. with 70% ethanol. Using paper partition chromatography (4), approximately 50μl. of each extract, spotted with a wire loop at the appropriate position on a sheet of Whatman No. 1 filter paper (18″ × 22″), were analyzed for amino-acids. The chromatograms were irrigated first in

[1] Manuscript received March 11, 1952.
Paper No. 2811 Scientific Journal Series, Minnesota Agricultural Experiment Station. Contribution from the Division of Plant Pathology and Agricultural Botany, University of Minnesota, St. Paul, Minnesota. Presented at the 36th Annual Meeting, May 1951.

one direction with phenol saturated with water in an ammonia atmosphere, and then, after drying for 24 hours at room temperature, in the other direction with a mixture consisting of equal parts of 2:4:6-collidine and 2:4-lutidine saturated with water.

Figure 1 is a semi-diagrammatic drawing of a chromatogram of moldy wheat showing the outlines of spots of ninhydrin-reacting substances present in the extracts. All the free amino-acids found in extracts of sound wheat were found in extracts of moldy wheat. Furthermore, gamma-amino butyric acid, spot No. 17, which was previously reported by Campbell *et al.* (2) to be present in agenized wheat flour but not in unagenized flour, was readily identified (3) only in moldy wheat extracts. Spot No. 12, an unknown, appeared in the same posi-

FIG. 1. Chromatogram of moldy Lee wheat. Spot No. 1, aspartic acid; No. 2, glutamic acid; No. 3, serine; No. 4, glycine; No. 5, threonine; No. 6, alanine; No. 7, glutamine; No. 8, histidine; No. 9, proline; No. 10, arginine; No. 11, lysine; No. 12, unknown; No. 13, valine; No. 14, leucine and/or isoleucine; No. 15, tryptophan; No. 16, tyrosine; and No. 17, gamma-amino butyric acid.

tion as a spot described by Wynn and Rogers (8) who found that a certain alcohol-soluble protein in blood plasma and serum advanced to this position when the same developing solvents were used. Cystine and methionine were detected in the extracts of both sound and moldy wheat using a method (4) in which these amino-acids are oxidized to cysteic acid and methionine sulfone, respectively, before chromatographic development. No phenylalanine was found in the sound and moldy wheat extracts; however, this amino-acid was found in a water extract of a first clear flour by Sullivan and Payne (7) who demonstrated the existence of free amino-acids in flour. After acid hydrolysis of the extracts with 6 *N* hydrochloric acid solution at 105°C. for 24 hours, spots corresponding to glutamine (No. 7), unknown (No. 12), and tryptophan (No. 15) no longer appeared on the chromatograms.

Visual comparisons of chromatograms of sound and moldy wheat, in respect to the size and color intensity of the spots, indicated marked changes in the concentrations of certain free amino-acids. Alanine, gamma-amino butyric acid, proline, serine, and lysine had apparently increased while histidine had decreased in concentration in moldy wheat relative to the concentrations of the other amino acids.

Further studies on "mold-free" wheat may indicate whether the changes found in the free amino-acid content of sound wheat were due mainly to seed respiration, mold growth, or both.

Acknowledgments

The author gratefully acknowledges the original suggestion of Dr. W. F. Geddes, Division of Biochemistry, for this research and the assistance of Dr. C. M. Christensen, Division of Plant Pathology, in determining the mold content of the wheat used.

Literature Cited

1. BOTTOMLEY, R. A., CHRISTENSEN, C. M., and GEDDES, W. F. The influence of various temperatures, humidities, and oxygen concentrations on mold growth and biochemical changes in stored yellow corn. Grain storage studies IX. Cereal Chem. **27:** 271–296 (1950).
2. CAMPBELL, P. N., WORK, T. S., and MELLANBY, E. The isolation of a toxic substance from agenized wheat flour. Biochem. J. **48:** 106–113 (1951).
3. CRUMPLER, H. R., and DENT, C. E. Distinctive test for α-amino acids in paper chromatography. Nature **164:** 441–442 (1949).
4. Dent, C. E. A study of the behavior of some sixty amino-acids and other ninhydrin-reacting substances on phenol-"collidine" filter-paper chromatograms, with notes as to the occurrence of some of them in biological fluids. Biochem. J. **43:** 169–180 (1948).
5. MILNER, M., CHRISTENSEN, C. M., and GEDDES, W. F. Grain storage studies V. Chemical and microbiological studies on "sick" wheat. Cereal Chem. **24:** 23–38 (1947).
6. MILNER, M., CHRISTENSEN, C. M., and GEDDES, W. F. Grain storage studies VI. Wheat respiration in relation to moisture content, mold growth, chemical deterioration, and heating. Cereal Chem. **24:** 182–199 (1947).
7. SULLIVAN, BETTY, and PAYNE, W. E. The amino acids of the water extract of flour. Cereal Chem. **28:** 340–342 (1951).
8. WYNN, V., and ROGERS, G. Observations on the behaviour of protein in paper partition chromatography. Austr. J. Sci. Res. (B) **3:** 124–131 (1950).

COMMUNICATIONS TO THE EDITOR

New Laboratory Methods for Drying Wheat Gluten [1]

DEAR SIR:

The conventional laboratory procedure for preparing small samples of dry wheat gluten consists of washing out the starch from dough by hand, dividing the gluten into small pieces and drying it in a current of air. The pieces of gluten tend to "case harden," thereby retarding the drying process with the result that the centre of the piece may undergo fermentation. Such gluten has impaired baking quality. The purpose of the present investigation was to devise more rapid methods of drying which would avoid deleterious effects on the gluten.

For efficient drying, gluten should be divided into relatively small pieces. Most methods of extrusion and shredding available in the laboratory give unsatisfactory results, and, in addition, the mechanical working of the gluten is harmful. Some preliminary experiments on grinding frozen gluten (−15°C.) in a Wiley mill were unsatisfactory because the surface material melted under grinding action. It was found, however, that gluten frozen in either liquid nitrogen or "dry ice" (solid carbon dioxide) became extremely hard and friable and could be readily milled into granular form. Two methods of drying were used: (a) freeze-drying; (b) air-drying at room temperature.

The procedure used with liquid nitrogen was as follows: pieces of wet gluten were carefully dropped into a wire basket submerged in liquid nitrogen in a Dewar flask. About 200 gm. of the wet gluten (65% moisture) were frozen in a period of approximately ten minutes. The frozen gluten was then quickly put through a Wiley mill equipped with a coarse screen (holes 3/16 in. diameter) and spread out thinly on a stainless steel tray. For freeze-drying, the loaded tray was transferred to the freeze-dryer and the drying operation started as soon as possible. A pressure of 2–3 mm. was maintained in the chamber and the gluten was held at a temperature of about −5°C. For air-drying it was found preferable to transfer the milled gluten to a stainless steel screen (60 mesh) and expose it to a current of circulating air.

The procedure for freezing with dry ice was as follows: slabs of "dry ice" were ground in the Wiley mill to produce a fine "snow." Alternate layers of the "snow" and wet gluten were placed in a Dewar flask and let stand for about 20 minutes. The entire contents of the

[1] Issued as Paper No. 140 on the Uses of Plant Products and N. R. C. No. 2784.

Dewar flask were then passed through the Wiley mill and spread out on a tray. Drying methods were the same as those previously described. Control samples were prepared from the same batch of gluten by drying in air by the conventional method and also by freeze-drying unmilled gluten frozen at −23°C.

The quality of the various dried glutens was assessed by baking tests. Each gluten was blended with a soft wheat flour (protein 8.2%) to produce a protein level of 13.5%. The samples were baked in duplicate using the malt-phosphate formula. Experimental conditions, drying time and baking data are given in Table I.

TABLE I

BAKING QUALITY OF WHEAT GLUTEN DRIED BY VARIOUS METHODS

Sample No.	Pretreatment and Method of Drying	Drying Time	Baking Quality			
			Loaf Volume [2]	Loaf Volume	Crumb [3]	
					Color	Texture
		hrs.	*cc.*	*cc./unit protein*		
Base flour			440	53.6	6.0	4.5
Flour [1]			535	43.5	9.0	6.5
Gluten 1	Dried in air	36–48	560	41.5	6.0	6.5
Gluten 2	Frozen at −10°F., unmilled, freeze dried	24–36	650	48.1	7.5	7.5
Gluten 3	Frozen in liquid nitrogen, milled, air dried.	12–16	585	43.4	6.0	7.0
Gluten 4	Frozen in liquid nitrogen, milled, freeze dried	14–16	610	45.2	7.0	7.5
Gluten 5	Frozen in dry ice, milled, dried in air.	12–16	595	44.1	6.0	7.0
Gluten 6	Frozen in dry ice, milled, freeze dried.	14–16	615	45.5	6.5	7.5

[1] Original flour from which gluten prepared (protein 12.3%).
[2] Necessary difference for 5% level of significance, 15 cc.
[3] The Grain Research Laboratory, Winnipeg, uses the following verbal description for both color and texture: excellent, 8.6–10.0; very good, 7.0–8.5; good, 5.5–6.9; fair, 4.5–5.4; poor, below 4.5.

The baking tests showed that all of the other drying methods tested yielded gluten superior to that dried in air by the usual method. Freeze-drying of the gluten, without any other processing yielded the best quality product. There was also some indication in the other methods used that freeze-drying was superior to air-drying. An interesting observation was that all freeze-dried glutens were almost white in color and easily pulverized in comparison with air-dried glutens which were yellow in color and horny in texture. The time of drying was shortest for the milled frozen glutens dried in air although freeze-drying of the same material took only slightly longer.

Choice of the most suitable method among those described will be determined by such factors as availability of liquid nitrogen, dry ice

and freeze-drying equipment. For ordinary laboratory purposes, the "dry ice" technique followed by freeze-drying is a simple and effective process. If time is not a major factor, freeze-drying of the unmilled frozen gluten gives the best product.

The author wishes to acknowledge the valuable technical assistance of Mr. W. G. Martin and Mr. A. E. Castagne of this laboratory; thanks are also due Dr. I. Hlynka of the Grain Research Laboratory, Winnipeg, for performing the baking tests.

G. A. Adams,
Division of Applied Biology,
National Research Laboratories,
Ottawa, Canada

March 13, 1952

The Carbohydrates of the *Gramineae*. The Constitution of the Water-Soluble Polysaccharides of the Grain of Barley (*Hordeum vulgare*)[1]

Dear Sir:

In 1882 O'Sullivan described the isolation and properties of *alpha* and *beta* amylan, polysaccharides extracted from barley flour with water (7). Apart from the work of Lindet (5), Piratzky (9), Preece *et al.* (10) and Meredith *et al.* (6), very little additional information about these substances has become available since this early work of O'Sullivan and this is particularly true of the chemical constitution and structure.

For some considerable time we have been carrying out a series of investigations into the chemistry and structure of the gums or hemicelluloses obtained from a number of cereal grains such as wheat, barley, rye and oats (2).

Part of this study has been concerned with an examination of the carbohydrate components of barley flour that are extracted with water at room temperature and precipitated from aqueous solution with alcohol. It is advisable to treat the barley flour with boiling 85% alcohol in order to inactivate the enzymes present (10). The crude polysaccharide (barley gum) has been shown by paper partition chromatography to contain glucose, xylose and arabinose, a result in agreement with those of others (5,6,7,10). Methylation of "barley gum" either directly or through its acetate provides a methylated

[1] Paper No. 2868, Scientific Journal Series, Minnesota Agricultural Experiment Station.

product which has been shown by extensive solvent fractional precipitation to consist of a mixture of three components:

(a) a methylated arabo-xylan, $[\alpha]_D - 160°$ in acetone
(b) a methylated poly-α-glucosan, $[\alpha]_D + 158°$ in acetone
(c) a methylated poly-β-glucosan, $[\alpha]_D - 9°$ in acetone

By means of paper partition chromatography, using either methylethylketone-water azeotrope (1) or butanol-ethanol-water (8) as the irrigating solvent and either NN-dimethyl *p*-aminoaniline (1) or *p*-anisidine (4) as the spray reagent, and the phenol-sulfuric acid method for the quantitative analysis (3), it has been shown that component (a) gives upon hydrolysis 2,3,5-trimethyl-L-arabinose, 2,3-dimethyl-D-xylose, 2-methyl-D-xylose and D-xylose in the molecular ratio of 1:12:4:2 respectively. Component (b) has provided upon hydrolysis a mixture of the same components as those obtained from methylated amylopectin, namely, tetra-, tri- and dimethyl-D-glucose in the molecular ratio 1:6:1 respectively. The third component (c) appears to be structurally related to cellulose since it yields upon cleavage only 2,3,6-trimethyl-D-glucose.

An examination of the acetate of the barley gum has shown that the pentosan corresponding to the methylated component (a) is best separated by adding ether to a solution of the crude mixture of acetates in pyridine.

Details of this work, and similar studies being carried out with the corresponding cereal gums from wheat, rye and oat grain, will be published later

K. A. Gilles
Division of Agricultural Biochemistry,
University of Minnesota,
St. Paul 1, Minn.
W. O. S. Meredith
Grain Research Laboratory,
Winnipeg, Canada
F. Smith
Division of Agricultural Biochemistry,
University of Minnesota,
St. Paul 1, Minn.

June 10, 1952

Literature Cited

1. Boggs, L. A., Cuendet, L. S., Ehrenthal, I., Koch, R., and Smith, F. Separation and identification of sugars using paper chromatography. Nature 166:520-521 (1950).
2. Cuendet, L. S., Dubois, M., Koch, R., and Smith, F. Unpublished paper presented to the 36th annual meeting of A.A.C.C., Minneapolis, Minnesota (1951).

3. Dubois, M., Gilles, K., Hamilton, J. K., Rebers, P. A., and Smith, F. A colorimetric method for the determination of sugars. Nature **168**:167 (1951).
4. Hough, L., Jones, J. K. N., and Wadman, W. H. Quantitative analysis of mixtures of sugars by the method of partition chromatography. Part V. Improved methods for the separation and detection of the sugars and their methylated derivatives on the paper chromatogram. J. Chem. Soc. 1702–1706 (1950).
5. Lindet, L. Chemie vegetale-les hydrates de carbone de l'orge et leur transformations au cours de la germination industrielle. Compt. rend. **137**:73–75 (1903).
6. Meredith, W. O. S., Bass, E. J., and Anderson, J. A. Some characteristics of barley malt and wort gums. Cereal Chem. **28**:177-188 (1951).
7. O'Sullivan, C. α and β Amylan: Constituents of some cereals. J. Chem. Soc. **41**:24–32 (1882).
8. Partridge, S. M. Filter paper partition chromatography of the sugars. 1. General description and application to the qualitative analysis of sugars in apple juice, egg white and foetal blood of sheep. Biochem. J. **42**:238–248 (1948).
9. Piratzky, W., and Wiecha, G. Zur kenntnis des amylans. Wochschr. Brau. **55**:97-100 (1938).
10. Preece, I. A., Ashworth, A. S., and (in part) Hunter, A. D. Cytolysis in germinating barley. I. Some barley and malt polysaccharides. J. Inst. Brewing **56**:33–40 (1950).

BOOK REVIEWS

Principles of Plant Physiology. By James Bonner and Arthur W. Galston, California Institute of Technology, Pasadena, California. W. H. Freeman and Company, San Francisco, 1952. vii + 499 pp. Price $5.50.

Cereal chemists desirous of an introduction to plant physiology will be pleased to have available a textbook for their field which treats the subject matter from the viewpoint of the modern biochemist. The text is clearly written in a concise, logical style, and is printed in easily readable type. Summaries at the ends of the chapters reemphasize salient points. Each chapter is accompanied by a list of questions, some of the general discussion type, others aimed at recalling specific facts discussed in the text. A brief bibliography also accompanies each chapter and includes chiefly reviews or monographs from which the reader can trace down the original work. Less than half a dozen original papers are cited. The index contains about 1500 entries.

Both authors of this textbook have made many original contributions—primarily biochemical—to the scientific literature. Both are specialists in the field of plant growth and development. As might be expected, the special interests of the authors are treated in a highly competent fashion. The six chapters (over 30% of the text) on chemical interrelations of plant constituents provide a unified picture of biochemical metabolism, representing the only textbook discussion even approaching an adequate synthesis of the voluminous literature on this phase of plant physiology at the level of the elementary reader. An outstanding feature of this synthesis is the series of chemical schemes for glycolysis, the tricarboxyllic acid cycle, a dehydrogenase-carrier-oxidase chain, and phosphorylation cycles presented in attractive, up to date, and appropriately simplified diagrams. The reader also is given an excellent introduction to plant growth phenomena emphasizing the hormonal aspects of this general subject. In spite of the fact that another 30% of the text proper is devoted to this subject, it is the reviewer's belief that the material is treated in no greater detail than should be considered adequate. However, in a text of less than 500 pages the inevitable result of an adequate consideration of one area is the neglect of another. Only 8% of the text is concerned with photosynthesis, 6% with mineral nutrition, 6% with overall water metabolism (noncellular), 2% with osmotic phenomena, 3% with various phenomena of permeability and ion accumulation, 5% with aspects of solute translocation, and less than 1% with cold resistance. The reviewer finds the almost casual neglect of so many important aspects of plant physiology a

most unhappy feature of this volume. These deficiencies will greatly impair the usefulness of the book.

Considering this book chapter by chapter certain noteworthy features stand out. The text is well organized. Following an introductory chapter orienting plant physiology in relation to applied fields and to other areas of botanical study, the main part of the book is divided into three sections: nutrition, metabolism (narrowly defined in the sense of chemical change), and growth. There appears to be no justification for this particular division although, with the exception of photosynthesis, the subject matter is arranged in a logical sequence. Placing the discussion of photosynthesis ahead of everything else was quite unfortunate. The reader with little previous background may find this chapter frustrating. While the topics of photosynthesis, mineral nutrition, and water economy are treated far too briefly to be satisfactory, soil water relations are handled somewhat more competently. Solute translocation is discussed appropriately and well. The chapter on enzymes is far too brief, but what it does contain is effectively presented. There is no adequate discussion of reaction kinetics as applied to physiological phenomena. Only two pages are devoted to a purely qualitative discussion of the mechanism of catalysis. The chapters on carbohydrates, respiration, nitrogen metabolism, lipids, and a catch-all chapter including a discussion of carotenoids, rubber, tannins, sterols, etc., are written as straight biochemical exposition. The discussion of respiratory metabolism is slightly marred by a lack of explanation for the dependence of respiratory rate on oxygen tension, the role of diffusion being ignored. Also, consideration of the Pasteur effect (not so identified) is limited to two sentences which give the reader an erroneous impression. The treatment of growth kinetics is superficial though the remaining chapters on growth physiology are generally excellent. Especially noteworthy is the authors' appraisal of the early work on the isolation and identification of naturally occurring auxins. Another departure from traditional views is the complete absence of any discussion of the "C/N ratio" and its relation to reproductive development. (Probably by now most plant physiologists recognize that C/N ratio is just an involved way of expressing nitrogen content). The reviewer found the discussion of photoperiodism one of the most delightful portions of the text. Various phenomena of dormancy are well presented but briefly so. Differentiation is treated rather narrowly and mostly from the viewpoint of the hormone physiologist. A very brief discussion, entitled Plant and Environment, constitutes the final chapter.

A general criticism of the book is that there is no consideration of the principles of plant physiology referred to in its title. If there are unifying principles for this area of botany, the reader is left to discover them for himself.

Aside from important omissions, the volume contains some surprising errors and half-truths. For example:

"There is no direct method for the measurement of turgor or wall pressure" (Page 89); cf. Arens. Planta **30:** 113 ('39).

Fermentation ". . . is carried out by higher plant tissues only when external conditions make respiration impossible." (page 232).

"In the final phase of nuclear division . . . the chromosomes are slowly transformed into the chromatin reticulum of the non-dividing nucleus" (page 331).

The modified Höfler diagram (page 84) is theoretically and experimentally incorrect as drawn.

Figure 2–15 implies that photosynthetic assimilation continues for a half hour after the plant is placed in darkness.

However, in spite of errors and omissions this textbook achieves a high standard of scientific accuracy.

In summary, *Principles of Plant Physiology* undoubtedly will be useful even though it is woefully inadequate in its treatment of many non-chemical aspects of plant physiology. The book errs as much in this direction as its predecessors (and still competitors) err in the direction of neglecting or treating badly the subjects of biochemical metabolism. The disproportionate emphasis in this book results partly from an attempt to keep the volume short rather than comprehensive. It confirms the reviewer's belief that a satisfactory modern textbook of plant physiology cannot be a short book and, if it is to be well balanced, it probably will be written by a panel of collaborators whose research interests are broadly distributed.

Allan H. Brown
Department of Botany
University of Minnesota
Minnespolis, Minnesota

Communication of Technical Information. By Robert M. Dederich, Chemonomics, Inc., New York, N. Y. 1952. 116 pp. Price $5.00.

As our technical knowledge grows in its range from the use of atomic tracers in plant research to new statistical tools, so the technician grows apart from his many audiences.

Concern over this technician-to-audience gap arose at a conference on industrial research at Columbia University and led Mr. Dederich to attack the problem at an advanced theoretical level. To his writing he brought academic training in literature and service as an engineering officer in the Navy in World War II.

The nature of this problem of communicating ideas and recently developed theories are first advanced by the author. He uses current research in psychology, anthropology and sociology.

Next, Mr. Dederich analyzes the process of communications in industry and sketches the major barriers to effective communication. He suggests solutions at the level of how one thinks about using words or symbols to convey technical information to various audiences.

The chapters on what-should-be-reported and what-should-be-written take nearly half the book and bring down to cases some of the theories of the opening pages.

In nine pages on "conferences" the problem is introduced for the undergraduate. However, the conference-hardened research man will not find as much reward here as in the remainder of the book.

A practical reader of the type who is helped by check-lists might sum up the theories as a series of questions on a single sheet. Then he might scan the questions as a reminder when (a) thinking about a technical information problem, (b) writing the information, (c) re-writing the information, or (d) explaining the report to some who did not understand—or who forgot.

By trying to apply such a list, one also would collect many examples to illustrate and illuminate the theories set out by the author. These theories, he would probably agree, are quite generally accepted and quite generally ignored. This behavior emphasizes the importance of an attack like Mr. Dederich's book, a new assault on this ancient barrier to the progress of science.

CHARLES E. SWANSON
Institute of Communications Research
University of Illinois
Urbana, Illinois

La fabrication industrielle des pâtes alimentaires. By C. Renaudin. Second edition, 405 pp. Dunod, Paris, France, 1951. Price, 2.450 fr.

The second edition of *La fabrication industrielle des pâtes alimentaires* is a technical book which covers the manufacture of macaroni products and describes the specialized machinery used in this industry. It contains 229 photographs, drawings and graphs.

The subject matter is divided as follows: (1) history, statistics, legislation; (2) general properties of alimentary pastes, their composition and nutritive value; (3) ingredients used in the manufacture of macaroni products: (a) semolina: its manufacture and quality control, (b) water: water softening systems, (c) dyes and other secondary products; (4) manufacturing processes: (a) preparation of semolina, (b) mixing, (c) kneading, (d) pressing, (e) continuous manufacturing process, (f) rolling, etc.; (5) drying of alimentary pastes: (a) open air drying, (b) the theory of drying and description of some modern drying equipment; (6) packing, quality control and storing; (7) description of a macaroni plant.

Although the book contains interesting and up-to-date information as well as stimulating discussions on the subject, the organization is rather poor for a technical treatise. This is especially troublesome in view of the absence of a subject index.

In spite of these limitations, this book will be of value to the manufacturers of macaroni products, and it will provide the cereal chemist interested in the field with useful information.

LOUIS S. CUENDET
Division of Agricultural Biochemistry
University of Minnesota
St. Paul 1, Minn.

The Merck Index of Chemicals and Drugs. 6th ed. Published by Merck and Co., Inc., Rahway, New Jersey. 1952. 1167 pp. Price $7.50 for the regular edition; $8.00 for the thumb-index edition.

The sixth edition of this well-known work continues the original purpose of providing "a concise, comprehensive and reliable encyclopedia of chemicals and drugs for the chemist, pharmacist, physician and members of allied professions." The fifth edition was published in 1940 and the current edition has been extensively revised and expanded. It contains more than 8,000 descriptions of individual chemicals, some 2,000 structural formulas drawn to conform with Patterson's Ring Index, and about 20,000 names of chemicals and drugs alphabetically arranged and cross-indexed. Several other features of the fifth edition have been retained in revised form; these include tables of indicators, coal tar colors, and a section on first-aid in poisoning.

New features include a table of standard buffers for calibrating pH measurements; a table of radioactive isotopes giving their half lives and type of radiation, and a table of current medical uses for radioactive elements and compounds. A new section lists more than 300 organic "Name" reactions with original and review references, together with a description and structural representation of each reaction. There is an up-to-date periodic table, a table of international atomic weights, and close to 150 pages of appendices on such subjects as thermometric equivalents, anti-freeze mixtures, refractive index of liquids, saturated solutions, percentage solution tables for apothecaries, and atomic weights and their multiples and logs.

Each main entry indicates the preferred chemical name or other chemical names, common or popular names, generic and trade names. Properties and sources of substances, preceded by methods of preparation, with literature references, are given, followed by an indication of their industrial, medical, or veterinary uses.

An indication is given of commercial availability and grades, such as U.S.P., N.F., and reagent. Empirical formulas, molecular weights, and percentage composition are given for all chemicals having a definite structure.

This authoritative encyclopedia should be on every chemist's bookshelf.

W. F. Geddes
Division of Agricultural Biochemistry
University Farm
St. Paul 1, Minnesota

Cereal Chemistry

EDITORIAL POLICY

Cereal Chemistry publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, or fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

Cereal Chemistry gives preference to suitable papers presented at the Annual Meeting of the American Association of Cereal Chemists, or submitted directly by members of the Association. When space permits, papers are accepted from other scientists throughout the world.

The papers must be written in English and must be clear, concise, and styled for *Cereal Chemistry*.

Manuscripts for publication should be sent to the Editor in Chief. Advertising rates may be secured from and subscriptions placed with the Managing Editor, University Farm, St. Paul 1, Minnesota. Subscription rates, $11.00 per year. Foreign postage, 50 cents extra. Single copies, $2.50; foreign, $2.60. Back issues, $3.00.

SUGGESTIONS TO AUTHORS

General. Authors will find the last volume of *Cereal Chemistry* a useful guide to acceptable arrangements and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" (*Trans. Am. Assoc. Cereal Chem.* **6**: 1–22. 1948) amplifies the following notes.

Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, and all original drawings or photographs for figures. If possible, one set of photographs of figures should also be submitted. Originals can then be held to prevent damage, and the photographs can be sent to reviewers.

Editorial Style. A.A.C.C. publications are edited in accordance with *A Manual of Style*, University of Chicago Press, and *Webster's Dictionary*. A few points which authors often treat wrongly are listed below:

Use names, not formulas, for text references to chemical compounds. Use plural verbs with quantities (6.9 g. were). Figures are used before unit abbreviations (3 ml.), and % rather than "per cent" is used following figures. All units are abbreviated and followed by periods, except units of time, which are spelled out. Repeat the degree sign (5°–10° C.). Place 0 before the decimal point for correlation coefficients ($r = 0.95$). Use * to mark statistics that exceed the 5% level and ** for those that exceed the 1% level; footnotes explaining this convention are no longer required. Type fractions on one line if possible, e.g., $A/(B+C)$. Use lower case for farinograph, mixogram, etc., unless used with a proper name, i.e., Brabender Farinograph. When in doubt about a point that occurs frequently, consult the Style Manual or the Dictionary.

For more detailed information on manuscript preparation see *Cereal Chem.* **29**: 89–90 (1952).

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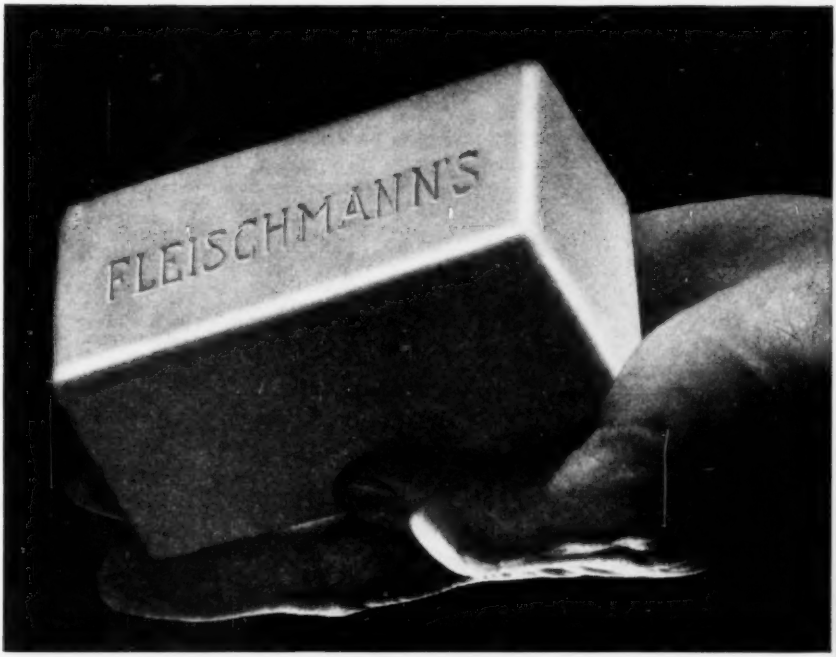

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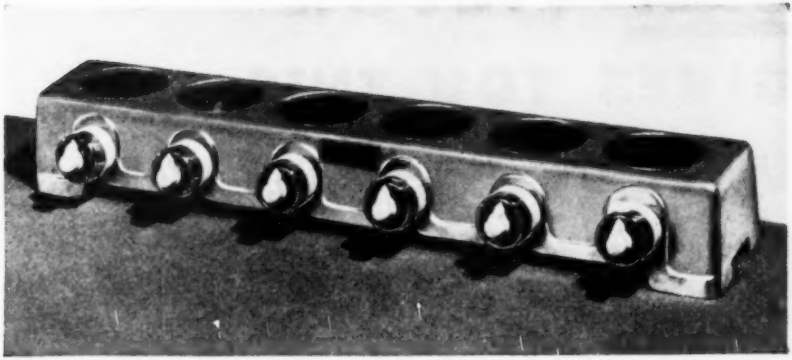

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